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THE REACTIONS OF PLANTS TO ULTRA-VIOLET

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GENERAL CONSIDERATIONS

It has been recognized for a considerable time that in general, as Ellis and Wells ('25) observe, there are two effects of ultra-violet radiations upon organisms, designated as stimulative and lethal, with the dividing line approximately at 2900 Å. If, however, various types of organisms and their different functions are considered separately both the exact significance of these terms and the limits of the regions concerned must be defined more explicitly. In addition, the numerous environmental factors so modify the effects of any one of them that unless the conditions are exactly stated there is a conflict of results such as has been emphasized by Popp and Brown ('33). It must be recognized, however, that such conflict of results is due to the different conditions under which the experiments have been carried on, the differences in physiologic action of various combinations of ultra-violet regions, and the specificity of organisms, rather than to a non-responsiveness of plants to ultra-violet.

The terms "stimulative" and "lethal" must be defined in relation to definite standards, and when we are dealing with a complex organism and especially with one of our higher plants

which is not so completely integrated as are the higher animals, we must recognize that these terms may be applied either to the whole organism or to one or more of its parts or functions. As is well known, the lack of a proper amount of light results in the etiolation of green plants. Under such conditions the use of ultra-violet irradiation cannot be expected to give a stimulation which will reveal itself in greater elongation. In fact the opposite result, as might be expected from the known action of the violet end of the spectrum, actually occurs. Under conditions of reduced visual illumination tomato plants irradiated with the mercury arc are shorter than those which are unirradiated, although with a moderate amount of irradiation from the mercury arc the dry weight of tissue produced may be the same as in the somewhat etiolated, unirradiated plants (table 1). More strongly irradiated plants under the same conditions develop less tissue as determined by its dry weight, and correlated with this reduced vegatative growth there may be much more abundant formation of green fruit.

TABLE I

REACTION OF TOMATO PLANTS TO ULTRA-VIOLET IRRADIATION UNDER REDUCED VISIBLE ILLUMINATION

	Set A*	Set B*	Set C*
Average increase in height in 2 months	28.9 cm.	21.6 cm.	20.9 cm.
Average dry weight	0.73 gm.	0.71 gm.	0.57 gm.
Weight of green fruit produced	(26 plants) 26.3 gms.	(26 plants) 23.1 gms.	(20 plants) 55.7 gms.

* Set A (30 plants) unirradiated controls. Set B (29 plants) and C (21 plants), irradiated at 50 inches from the mercury vapor arc through a water filter of Vita glass, 9 minutes and 18 minutes daily, respectively.

Thus it is evident that the action of ultra-violet on these plants may result at the same time in reduction of elongation and in an increase in fruit formation. On the other hand, a reduction in the amount of ultra-violet attained by increasing air absorption through greater distance, concurrent with a

more normal amount of visible illumination as evidenced by lack of etiolation in checks, results in actual increase in dry weight, elongation, and mineral content (Fuller, '31). Similar results have been obtained in other series of experiments in our laboratory more recently and have also been reported by others (Benedict, '34), all of which indicate clearly that the criteria of "stimulation" and "injury" vary with the species and with the different conditions under which they are studied. We cannot determine at the present time how much of the effect is due to direct action and how much to indirect. Thus it is probable that the greater fruit formation in plants showing strongly retarded vegetative growth is due largely to the disturbed nutritional balances as may be inferred from the work of Kraus and Kraybill ('18), Murneek ('26), and Harvey ('31), yet some direct stimulation is possible. As will be demonstrated in later papers in this series, various phases of respirational activities react differently to the same ultra-violet regions. Enzymic activity may be stimulated as shown by Fuller ('32), v. Euler and Günther ('33), Bersin ('33), and from studies to be reported in a later paper in this series, and this may occur when there is coincident evidence of injury to other systems.

It is especially important therefore to examine the applications of our criteria in order to avoid much needless discussion of results based upon uncertainty in their use and to determine the conclusions which may properly be drawn. In the study of the effects of radiations upon organisms many indefinite factors exist, not the least of which is the variety of material used in the experiments. It is now well understood that while there are certain broad fundamental ways in which all green plants are similar in their physiology, yet each species and variety has a physiology of its own both qualitatively and quantitatively different from that of any other. We do not know as yet the experimental limits of this specificity in any given case except as expressed in the general results which give us the indistinct concept of the species. This one variable factor has given rise to many pages of useless discussion in the papers

on radiations. To expect all plants to respond uniformly to such a complex environmental factor as ultra-violet is to ignore their specific character as well as the specific action of different types and quantities of energy.

Another general factor which frequently is forgotten is the historical, evolutionary development of the plant kingdom. The physical concept of a smooth cumulative curve of effects from a given kind of force, such as increasing heat causing an increasing expansion of an iron bar, has restricted value when applied to plants. They have been subjected through eons of time to circumscribed ranges or intensities of certain physical and chemical conditions to which they have become adjusted. Therefore in subjecting them experimentally to the entire range of a given force we should expect them to respond in one manner in the range to which they have become adjusted and in another manner in the ranges outside of their historical experience. Thus we find that within a certain restricted range of temperature most plants increase in size in a direct ratio to increased temperature, while outside of this range they react quite differently. It is necessary of course to take into consideration in such a statement a certain elastic adaptability which enables living organisms to extend their responses somewhat normally even beyond the usual range of the physical factors. These two variables, plant specificity and specific adjusted reaction to certain ranges of each environmental factor, are probably more important in influencing attempts to obtain generalizations than many other factors which have been so greatly stressed by some.

As concerns growth related to any external stimulus, there are evidently three possible general conditions: (1) it may be definitely increased over that under established standards due to dominance of stimulative over retarding influences; (2) it may be definitely reduced below the established standards, due to dominance of retarding factors over stimulative ones; and (3) there may be such a balance of these two sets of effects that no significant increase or decrease can take place. It is possible also that in this last case certain physiological activities

which are not reflected in growth may be stimulated or retarded. From the above considerations it is clear that growth cannot be a complete index of stimulative or lethal reaction of plants.

In the field of radiations growth as a criterion of stimulation must be carefully defined. Size, wet weight, dry weight, and number of leaves must all be given careful consideration and balanced against one another, for no one of them can be used exclusively to indicate stimulation. In the phase of reproduction radiations are coming to be recognized as more and more important, and we expect that stimulation will be evidenced in the number, form, and size of the fruiting structures at times when vegetative features fail to give evidence of reaction. Occasionally the phrase "beneficial effects" (Popp and Brown, '33) is used more or less as synonymous with stimulation, but it is liable to have a subjective rather than an objective content, and a very careful assessing of the values of the various stimuli and of criteria must be made before it can have a quantitative value. Thus a stimulated growth may be beneficial under certain circumstances and stages of growth, but positively injurious at other times, while the reverse may also be true. Let us now consider some of the ways in which growth has been used as a criterion in radiation experiments on plants.

Experiments, such as those of Popp and Brown ('28), in which the early seedling stages have been used as a test for stimulation, can hardly be used as a proof against the concept of stimulation as applied to older plants. It is a well-known fact in general physiology that the physiology of the young organism is often quite different from that of the more mature individual. Moreover, the growth curve of an individual shows an exceedingly rapid rise during the early seedling stages, and it is doubtful whether at this time all stimulative factors could be expected to affect the rate of growth even if they could at a later, slower period. On the other hand, retarding and limiting factors may become especially effective during the period of normally active enlargement. Thus a small decrease in the water supply at such a time may have a much greater effect

than at a later stage and hence have a greater antagonistic action toward the tendencies of reaction to stimulative factors.

It is entirely probable that one of the reasons why growth is so active during the early seedling stages is that there is stored away in the seed a maximum amount of all sorts of food substances, including the vitamines, stimulative to rapid growth. As a seedling matures it tends to exhaust the stored supplies, or distribute them throughout more and more tissues, or remove them from further utilization by combining them in more or less stable, permanent compounds unless renewed by favorable physiological processes. If, as may well be, the stimulative action of ultra-violet radiations is due to the manufacture in the plant of stimulative compounds, some of which may be stored in the seed, adequate to the maximum stimulation of the developing seedling, the stimulative action of ultra-violet would not become manifest until after the essential exhaustion of the seed reserves. For a similar reason, if plants are growing under daylight conditions in which ultra-violet adequate to maximum stimulation for a given set of conditions has been supplied naturally, then additional raying from an artificial source would not be evidenced in stimulation. Hence experiments performed in one season of the year may not be at all comparable with those at another season, and likewise experiments under different aerial conditions such as arise from varying quantities of smoke, moisture, cloudiness, etc., cannot be fairly compared, nor equal value attached to negative results as to positive ones.

As illustrating the seasonal effects upon ultra-violet the following may be cited: In summer about 1 per cent of solar radiation is in the ultra-violet and about .04 per cent in the region 290 mμ to 320 mμ. In winter the figures are .2 per cent total ultra-violet and .013 per cent in the region 290–320 mμ (Laurens, '33, p. 54). While these values were obtained for a different region than ours, comparisons of numerous other determinations indicate that these are fair approximations for many other regions.

It has been our common experience in this laboratory that even injury from irradiation by an unscreened mercury vapor

arc is much more difficult to demonstrate on tomato plants which have developed during the summer months in the greenhouse than upon those developed during the winter months. Older plants in the summer are likewise less easily injured by ultra-violet than younger ones. Some of these differences may no doubt be attributed to certain anatomical changes which occur in plants when rayed with ultra-violet such as described by Eltinge ('28).

From the various considerations just discussed it is evident that experiments showing lack of stimulation under ultra-violet cannot be used to condemn positive results unless it is certain that the other environmental and biological conditions have been duplicated. If one compares the investigations reporting lack of stimulation with those most carefully controlled which report stimulation (Fuller, '31; Benedict, '34), it is evident that no careful attempt has been made in the former to duplicate the conditions under which stimulation has taken place. It should be pointed out here that there is a decided difference in the conditions of irradiation between those in which there is given a long-time or close exposure of seeds, seedlings, and more mature plants to artificial irradiation sources and those in which short-time or long-distance exposures are made. Short injurious ultra-violet waves are much more fully absorbed by air than are the longer ultra-violet, and in short-time exposures the variable of visible light is eliminated in a practical manner and the variable of infra-red is also greatly reduced. Although both of these variables have been stressed recently (Popp and Brown, '33), as seriously influencing conclusions concerning the ultra-violet stimulation, it should be noted that there is a great difference between adding a little more visible light and some extra heat to the environment of a plant well supplied with these factors and adding ultra-violet to an environment almost devoid of such a factor. Shirley ('29), for example, records that the rise in temperature due to the heat from his 1500-watt lamps at 24 inches distance was less than 0.5° C., while Fuller's lamp was at 100 inches and a large electric fan was constantly used during irradiation to remove so far as possible any excess heating.

Since for all of the experiments reported from our laboratories the plants were grown in the winter or spring in the greenhouses, and much of the ultra-violet which is accepted as stimulative in character is screened out by greenhouse glass and reduced by climatic and seasonal conditions, it is evident that the plants would be in a condition to be stimulated by the ultra-violet from an artificial source. Such actual increased growth has been recorded in our laboratories for a variety of plants irradiated by a mercury vapor arc. Such distances and such screens were used as would remove injurious ultra-violet, and essentially eliminate the variables of added visible light and of infra-red since they constituted but a minute fraction, an average of nine minutes a day exposure, of the total of such energy received by the plants during the experimental growth period. In these experiments (Fuller, '31) where there were ten sets of one hundred plants each, both the wet weight and dry weight of all the sets rayed through filters which cut off the injurious ultra-violet were significantly greater than those of the unrayed controls. In six out of the eight rayed sets also significant increases in height occurred.

The pertinent question may be raised as to how one may explain the stimulative action of small amounts of ultra-violet on organisms when we are inclined to discount the larger amounts of visible and infra-red radiations from the same artificial source. It is rather generally accepted that within certain limits growth increases quantitatively with light and heat. On the other hand, the changes in plants caused by ultra-violet seem to be qualitatively different from those caused by other radiations (Jacobi, '28) and certainly cannot be estimated in terms of the quantity of energy available in the ultra-violet compared with that present in visible and infra-red radiations. Or we may say that a small quantity of energy added experimentally is more effective in this range of radiations than in the others, due perhaps to a more complete absorption and to the fact that the amount of ultra-violet added is proportionately very large during winter and early spring. Whether, as has been suggested (Laurens, '33), these shorter

wave lengths cause special intimate molecular or atomic rearrangements we cannot at present know. It may be also that something akin to the rigor or tetanus produced in organisms by consecutive applications of stimuli might prevent stimulation in the presence of larger amounts of the same forces, but in the absence of natural ultra-violet of certain wave lengths an artificial application might be especially effective, particularly when added in successive, short doses. Johnston ('32) has recently used infra-red radiations in conjunction with light and found that when the latter was well below the optimum for tomato plants the near infra-red stimulated growth, but with a higher degree of illumination the infra-red stimulation was much less. This seems to indicate that stimulative action of the infra-red is much less effective as normal illumination is approached.

In conclusion we may summarize the discussion as follows: Physiological response includes both increased and decreased activities. Growth is a resultant of numerous physical and chemical reactions. Stimulation or increased growth may therefore be a resultant of some increases and some decreases of physical and chemical activities. Injury and retarded growth may indicate either a total resultant reaction of all the physical and chemical reactions or interference with some one or several specific reactions. Ultra-violet and X-radiations cannot be expected to affect every one of the numerous physical and chemical activities in an accelerative fashion or, on the other hand, all in a retarding fashion. Evidences are found all through the numerous studies on radiations to substantiate these statements. It is to be expected, therefore, that there will be numerous apparently contradictory results as long as growth alone is taken as a criterion for stimulation and interpreted as the result of purely quantitative applications of energy. This is especially true if the results from studies upon different species and varieties are massed together and compared. It is evident therefore that experimental studies which have failed to produce acceleration of growth cannot be used to impugn those which have demonstrated stimulation.

The element of critical surprise evidenced in Popp and Brown's ('33) summary of ultra-violet work, that such contradictory results are published, and the implication of untrustworthy data are not wholly justifiable in view of the considerations discussed above. We should expect just such a condition, and instead of attempting at the present time to amalgamate all such work into a consistent unit and to evaluate it in terms of uniformity of growth results, it would be more profitable to recognize the existence of numerous separate problems and allocate to each the data properly belonging to it. In some of the earlier work where only a reconnaissance of the field has been attempted conditions have not been completely controlled, and it is indeed true that in other studies unjustifiable deductions have been made, or poor technique has made the work essentially valueless as soon as more carefully controlled experiments are reported. Finally, the historical development of the plant kingdom has led to adjustments of plants to restricted ranges of environmental factors. Within these ranges any green plant will react quite differently than it will outside of them, and therefore even opposite reactions may be expected from the same plant in these different ranges, as, for example, stimulated growth in some radiations and retarded in others.

A series of investigations has therefore been initiated and is in progress in which separate physiological activities are being studied in relation to ultra-violet, in an effort to define the conditions under which stimulative and lethal actions occur.

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STUDIES IN ULTRA-VIOLET AND RESPIRATORY PHENOMENA. I[1]

Review of Work Published before June, 1935

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I. Introduction 772
II. Basal metabolism of animals 773
A. Lower forms 773
B. Vertebrates 775
1. Entire organisms 775
2. Individual tissues 778
3. Biochemical studies 779
C. Human subjects 781
1. Irradiations from artificial sources 781
2. Irradiation as a climatic factor 784
D. Theoretical considerations 787
III. Metabolism of plants 790
A. Fungi 790
1. Bacteria 790
2. Botrytis 792
3. Yeast 793
B. Green plants 800
C. Theoretical considerations 801
IV. Oxidizing enzymes 802
A. Catalase 802
1. *In vivo* studies 802
a. Animals 802
b. Plants 805
2. *In vitro* studies 805
3. Theoretical considerations 810

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B. Oxygenase 812
1. Animals 812
2. Plants 812
3. Theoretical considerations 813
C. Tyrosinase 814
1. Animals 814
2. Plants 814
3. Theoretical considerations 814
D. Peroxidase 815
1. Animals 815
a. Milk 815
b. Tissue 816
2. Plants 816
a. *In vivo* studies 816
b. *In vitro* studies 817
3. Theoretical considerations 819
E. Dehydrogenase 820
1. Animal tissues 820
2. Plants 821
a. Bacteria 821
b. Higher plants 821
3. Xanthine dehydrogenase 822
4. Theoretical considerations 824
V. Sulphydryl compounds 824
A. Experimental 824
B. Theoretical considerations 826
VI. Conclusions 826
VII. Bibliography 827

I. Introduction

The study of the relationships between ultra-violet light and biological materials has become enormously extended in recent years. The nature of the problems treated is so diverse that the field is already breaking up into smaller divisions, each of which is expanding so rapidly that it fully occupies the energies of students who wish to keep abreast of the developments. As soon as any field of research reaches these proportions, the need of critical reviews and extensive published bibliographies becomes increasingly apparent.

The present paper is a critical review covering the effect of ultra-violet radiation on respiration phenomena. It is scarcely possible that no paper has been overlooked, yet it is felt that the bibliography is essentially complete. Only papers giving data indicating the significant presence of ultra-violet light

have been included, and many studies on sunlight and on light from indefinite sources have not been mentioned.

Reviews of ultra-violet physiology containing material on respiratory phenomena have been published by Euler and Lindberg ('12), Pincussen ('21a, '30), Meyer ('26), Lippmann ('28), and by Laurens ('33), all of which are either outdated or inadequate. The best general review, especially from the viewpoint of biological theory, is that of Pincussen ('30), while the most recent work is that of Laurens, which, because of its wide scope, cannot give an adequate survey of all the pertinent literature.

II. Basal Metabolism of Animals

A. Lower forms

Merker ('25a) was the first to report the respiratory activities of irradiated lower animal forms. In his earlier experiments he used a 1000-watt filament lamp, and although he makes no mention of ultra-violet rays, a light operating at such a wattage must have emitted a considerable amount of the longer ultra-violet. Merker was primarily interested in the problem of photosensitization by fluorescent substances. He treated living earthworms *(Lumbricus terrestris)* for several hours with a .001 per cent neutral-red solution, and then rayed them in a constant-temperature water bath. By determining the loss of dissolved oxygen from the water inhabited by the organisms, he calculated the oxygen consumption and found that it was markedly increased, but he concluded that the metabolic effects of the increased movement of the organisms when rayed could not be separated from the specific effect of irradiation. Later ('25b) he repeated these experiments with *Allolobophora caliginosa,* but extended the period of irradiation to 6 hours. An increased use of oxygen was evident for the first 200 minutes, after which there was a decrease. These worms lived until the next day, but showed definite injury. In yet another publication ('25d) these results are duplicated, and in the following year ('26) apparently the identical data were republished.

These papers were followed by another (Merker and Braunig, '27), which reported the results of raying a number of species of moist-skinned animals with the quartz mercury arc at a distance of 18.5 cm., for various periods. Both *Allolobophora* and *Lumbricus,* which were rayed for about 20 minutes, showed a decided decrease in oxygen use, and the raying of *Gammarus pulex* L., a swimming amphipod crustacean, for 54 minutes resulted in a very great decrease. In all cases of injury to the oxygen consumption, the termination of the irradiation allowed the return to about the normal rate. This return to normality was temporary, showing that the organisms had been permanently injured. Studies with filters showed that the injurious region was between 3660 Å and 3840 Å and that red light, 7800 Å to 6500 Å, had no effect.

Merker's experiments were not subject to definite interpretation because the organisms were stimulated to active movement, but this difficulty was satisfactorily met by Sonne ('26, '29). He used the pupae of meal worms, which show no movement when irradiated and have no disturbing food factors. In his earlier paper, he reports that the unscreened mercury arc produced a 40 per cent increase of oxygen use with an exposure of 45 minutes, and that the pupae turned black and died in a few days. Pupae rayed under a glass filter or through solutions of methylene-blue or potassium bichromate apparently were uninjured, although the oxygen stimulation was 20, 10, and 0 per cent, respectively. The respiration of excised tissues responded to heat, as did living tissue, but there was no response to irradiation. Sonne concluded that the action of light must depend on the presence of nervous and vascular tracts, and hence he made the unwarranted assumption that mineral metabolism must have been concerned in some way.

This work was carefully repeated three years later. The pupae rested on a thermo-needle which indicated any rise in temperature during the raying. The temperature of the water bath was so varied that no significant temperature change occurred in the organism. The open flame of a Kromayer lamp was used, as well as various filters, the irradiation lasting 30 minutes to 1 hour. The full arc gave a 40 per cent increase in

the oxygen uptake, a blue filter gave a 10 per cent increase, and a glass filter passing the visible and near ultra-violet gave an increase of 20 per cent. Sonne ('29) stated again that an increase in metabolism must bear some relation to the beneficial effects of raying on mineral metabolism. There is no evidence to support this assumption, for, as will be shown later, there is no undoubted metabolic change of human patients when rayed with the usual therapeutic doses.

Wickwire and Burge ('27) studied the effect of irradiation on the sugar assimilation of *Paramecium caudatum*. They used a Cooper-Hewitt mercury arc, operating on 170 volts and 3.5 amperes. Five cc. of the washed and centrifuged organisms were added to 100 cc. of a .1 per cent solution of dextrose, levulose, or galactose. The suspensions were rayed for 10 hours at various distances, after which the amount of sugar remaining in the solution was determined. When the raying was carried out at a distance of 50 cm., all the cells were killed and the sugar use was lowered 80 per cent. When rayed at 400 cm., the lowering was 35 to 40 per cent. In the latter instance, the cells were as active as the controls and appeared to be uninjured. In view of the fact that the activity of insulin is destroyed by ultra-violet light, these authors added insulin periodically to the solution and found that the lowering of the sugar use was prevented. It was concluded that the ultra-violet light affected the Paramecia by destroying their insulin. Absorption spectra showed that the cells absorbed some of the shorter waves, but that no bands were completely removed. Ultra-violet is absorbed so variously by organic substances that no physiological significance can be attributed to the specific absorption that these authors obtained.

Roskin and Schischliajewa ('34) obtained irregular results when they studied the effect of ultra-violet light on the oxygen uptake of *Paramecium*, although they concluded that in general the oxygen consumption was lessened.

B. VERTEBRATES

1. Entire organisms.—Various vertebrates were studied by Merker in connection with the problem of photosensitization.

He treated ('25a) the overwintered larvae of *Alytes*, a genus of obstetrical toads, the summer carp, and "Schlammpeitzger," an indefinite term for the Cobitidae, an Old-World family of small fishes, for several hours in .001 per cent neutral-red, and found that the illumination by a 1000-watt light gave notable increases in oxygen use. In a series of papers he reported this generally true. However, irradiation from the mercury arc always gave an important decrease in oxygen uptake. The paper by Merker and Braunig ('27) describes experiments with *Gammarus pulex* (young white fish), *Gasterosteus aculeatus* (a stickleback fish), *Rana esculenta*, and larvae of *Molge cristata* and *M. taeniata* (both belonging to the Old-World salamanders).

Harris ('25a) rayed rats in a small quartz respiration chamber with either a mercury arc operating on ½ kilowatt, or a carbon arc operating on ¼ kilowatt. He used the full arc, the visible only which he obtained by a plate glass filter, and ultra-violet only obtained by a "Blue Uviol" filter which passed rays between 2910 Å and 4360 Å. A constant temperature was maintained and the carbon-dioxide elimination was estimated at 15-minute intervals with an accuracy of ± .5 per cent. The results were calculated in terms of mgms. of carbon dioxide eliminated per square cm. of body surface. In one experiment neither the unscreened arc nor the visible alone gave a significant effect, but ultra-violet produced a definite stimulation. This author concluded that visible light nullified ultra-violet stimulation by a physiological antagonism, and not by any physical interference, and he made the interesting comment that this is the reason that human subjects suffer less in sunlight than when irradiated by equivalent dosages of pure ultra-violet.

It is not possible at the present time to conclude definitely concerning the physiological antagonism of different wave lengths of light. This phase of the problem must wait for the fuller development of monochromatic studies.

The work of Harris was questioned by Campbell ('26). He studied mice and rats in a constant-temperature chamber. The

unscreened illumination of a Cooper-Hewitt mercury arc, operating on 110 volts and 3.5 amperes, at a distance of 25 cm., was used for the first 1½ hours, a "Blue Uviol" filter for the next 1½ hours, and then an opaque screen for the next similar period. He found no metabolic effect, either immediate or delayed, of any of these spectrum regions.

The question was reopened by Crofts ('28a), who criticized previous students because they did not keep the rayed animals under observation for a sufficiently long period after the irradiation. She used canaries, because of their marked physiological sensitivity, and observed their oxygen use for a period of 6 days after irradiation. A Hanovia mercury arc was the source of illumination, at a distance of 25 cm., for a period of 10 minutes, this being equivalent to 14 lithopone units. The feathers were removed from the under side of the body to expose the skin. There was usually an abrupt drop in oxygen use immediately after raying, which averaged about 14.1 per cent. Single heavy doses caused a belated rise after the preliminary drop, averaging 21.6 per cent. Still heavier initial doses extended the period of depression. Daily rayings gave the usual depression, but no subsequent rise. It is particularly significant that there was no relationship between size of dosage and the amount of depression or elevation. This agrees with the present authors' results on yeast, which is reported in the third paper in this series, but it is improbable that similar mechanisms are involved.

Hardy ('28) extended the work of Crofts to rabbits, and increased the period of observation to 12 weeks. She used single doses of 24, 60, and 90 lithopone units, and also daily doses of 20 and 30 lithopone units which gave a total of 120 and 180 lithopone units respectively. She found no effect on the resting metabolism.

During the same year, Vasetko ('28) rayed white mice for 3 minutes with a mercury arc of the artificial sun type at a distance of 30 cm., and measured the heat production in a calorimeter for 10 hours. The heat production increased slightly. No observations were made on gas exchange.

2. Individual tissues.—The first observation on the effect of ultra-violet on the respiration of individual tissues was made by Gottschalk and Nonnenbruch ('23). They ground 2 gms. of frog muscle in 10 cc. of water to which .2 gms. of m-dinitrobenzol had been added. The preparation was rayed with a mercury arc at a distance of 70 cm. for periods of 15 to 90 minutes. Exposures of 45 minutes or less increased the oxygen use, the maximum stimulation of 24 per cent occurring with 30-minute exposures. Dosages of 60 minutes or more were depressing.

Wohlgemuth and Szörényi ('33c) placed the tissues of liver, kidney and skin in Ringer's solution, and studied the gas exchange by the manometric method of Warburg. A 75-watt lamp was suspended in the water bath 10 to 12 cm. from the vessel containing the tissue. In the absence of photosensitizers there was either no effect on the oxygen absorption or a slight depression, but in the presence of haematoporphyrin, there was a well-defined stimulation. This photosensitized fraction of the oxygen uptake apparently was not normal respiration, since it was not affected by temperature and since M/300 KCN not only did not inhibit it but actually had a stimulatory effect. Other experiments were carried out with a carbon arc drawing 30 amperes and with a quartz mercury arc. In these instances the light was removed 50 cm. from the vessels containing the tissues. In the presence or absence of sensitizers (haematoporphyrin or Bengal-rose) there was a decrease of the oxygen use. The sensitizers alone caused a decrease of 6 to 22 per cent, but when rayed with sensitizers the decrease was 17 to 43 per cent.

Guinea pigs that had been injured by raying usually showed an increased tissue respiration of the excised organs, and also occasionally an increase of anaerobic glycolysis. The authors postulated that these effects were probably due to the formation of toxic substances during the raying. They also ('33a) studied the effect of raying normal tissue of liver, kidney, skin, brain, and retina of guinea pigs. In the presence of sensitizers, irradiation with a carbon arc decreased oxygen use, probably because of a photo-oxidative destruction of protoplasm. If

sensitized guinea pigs were rayed until they showed acute photo-dynamic injury, the excised tissues showed an increased oxygen use, probably due to the formation of a toxic substance.

Since Ellinger had shown that ultra-violet irradiation changed histidin to histamin both *in vitro* and *in vivo*, Wohlgemuth and Szörényi (33b) attempted to show that histamin would call forth the same physiological effect as irradiation. Histamin stimulated the oxygen use by kidney, liver, brain, and skin, but Jensen sarcoma was unaffected.

3. Biochemical studies.—Pincussen ('21a) attempted to show an analogy between the action of ultra-violet on oxalic acid *in vitro* and *in vivo*. If oxalic acid is destroyed *in vivo*, then determinations of this substance in the urine can give no picture of oxidation within the body, and this uncertainty is further increased by the doubtful significance of its presence in the urine. In studying this problem, Pincussen injected oxalate subcutaneously into the rabbit, and then rayed the animal with a carbon arc. In control animals, 75 per cent of the added oxalate was recovered in the urine, but when the animals were sensitized by eosin or by the sodium salt of dichloranthracenedisulphonic acid, only about 45 per cent of the oxalic acid could be recovered. The conclusion was that oxalic acid was an end product of normal metabolism, and that irradiation caused the oxidation of the acid. By chemical analogy, the end product of the raying must be carbon dioxide and water.

Biochemical changes connected with metabolism were studied particularly by Pincussen and Zuckerstein ('29). Guinea pigs were fasted several hours, with and without previous eosin injections, then rayed, and killed 30 minutes later. A study of the individual organs showed that fat markedly increased in the serum but cholesterin did not change, while in the heart and liver, fat decreased but cholesterin increased. Eosin injection increased the magnitude of these effects. In the kidney, fat changed but little and cholesterin increased. The previous injection of eosin diminished cholesterin. In muscle, there was but little change in the absence of the eosin, but with it present fat was lowered.

In a further study of the biochemical changes in separate organs, Pincussen and Kawakami ('29) used a mercury arc operating on 140 volts and 4 amperes. Rats were rayed at a distance of 15 cm. for 30 minutes, after which they were killed and the organs removed. The liver and muscle showed an increase in glycogen and a decrease in lactic acid. The tables included in their paper show that the ratio, glycogen: lactic acid, is increased in the liver about 55 per cent and in the muscle about 45 per cent. In the heart no significant difference was found. The increase of carbohydrate in the organs of rayed animals had already been reported by Jacoby. The change in the glycogen: lactic acid ratio involves, according to these authors, a lower catabolism of carbohydrate, and probably also an increased synthesis, and the relation of these results to diabetes was suggested. Raying induces for a short period a small increase of blood sugar followed by a decrease. In diabetics, the sugar in the urine is also decreased, this being explained not by an increased oxidation of sugar but by its retention and storage in the liver and muscle as glycogen.

In later experiments, organs were removed from normal rats, minced, suspended in a thin layer, and then rayed with a mercury arc. The fact that results were not consistent indicates the possibility that the specific reaction of living animals to ultra-violet might not be due to the action of the irradiation as such, but rather to secondary reactions due to a generally disturbed metabolism.

Wohlgemuth and Szörényi (33a) reported that ultra-violet increased the anaerobic lactic acid formation in the absence of sensitizers, but diminished it in their presence. The source of radiation was either a carbon arc or mercury arc. Anaerobic glycolysis by the tissues of guinea pigs in the presence of sensitizers was shown ('33c) to be injured by intensive raying with a carbon arc. In the absence of sensitizers the glycolysis reaction was increased. Since ultra-violet irradiation had been shown to change histidin to histamin *in vivo,* they ('33b) studied the effect of histamin directly. Although tissue respiration was increased, anaerobic lactic acid formation was not changed.

C. HUMAN SUBJECTS

1. Irradiations from artificial sources.—Hasselbalch ('05) was the first to record the effect of ultra-violet on human metabolism. This worker rayed himself under carefully controlled conditions with a carbon arc, operating on 65 volts and 150 amperes. Relatively heavy exposures of about 1 hour were used, with the result that the frequency of inhalation was lowered, but the amplitude increased, the total ventilation therefore remaining unchanged. This condition may last for several days, and frequently longer than the erythema.

Durig, Schrötter, and Zuntz ('12) also found a slower rhythm of inhalation. The effect on actual metabolism was not definitely demonstrable, although their tables do show an increased use of oxygen by some subjects.

The results of raying patients suffering from various heart diseases were reported by Rubow and Sonne ('12). The carbon arc caused reduced frequency of inhalation and an increased amplitude, which were progressive in magnitude as the daily rayings continued. These results agree with those obtained by Hasselbalch on healthy patients. Rubow and Sonne observed that the development of erythema was necessary for the changes in inhalation and that super-sensitive patients who developed erythema with 5- to 10-minute exposures exhibited the typical reactions.

These data were verified by Austgen ('19), who rayed tubercular patients twice a week with the mercury arc at a distance of 70 cm., the dosage being increased from 3 to 20 minutes at each exposure. His results were very irregular. Of 70 patients, 35 showed no change, in 21 the rhythm of inhalation was increased, and in 14 it was lowered.

Ederer ('22) also studied this problem and reported that short exposures to the mercury arc had no effect on alveolar carbon-dioxide tension, but that moderate doses increased it, and large doses lowered it. These effects were found to be independent of the development of erythema.

Kestner, Peemöller, and Plaut ('23) observed an increase in oxygen use unless erythema developed. Their subjects had

fasted 12 hours, and were then rayed with a mercury arc at a distance of 50 cm. Animals with hair showed a reaction similar to the human subjects who developed pigment. These authors point out that the increase in oxygen absorption depends on the activity of the chemically active rays, and on the absence of heat rays. Since the application of heat to warm-blooded animals decreases their heat production this factor would counteract any stimulatory effect of the ultra-violet. This problem will be discussed more fully under the topic, "Theoretical considerations."

Several papers by Kestner and his co-workers ('23, '25, '26, '27) reported increased metabolism due to intense sunlight. Contrary to Kestner, Kroetz ('24) found that even though erythema always developed physiological changes occurred. The blood showed a temporary acidosis, followed by an alkalosis which was maintained for about a day. He attributed a stimulation of the respiratory center to the general effect of raying the organism. The presence of eosin did not influence these results.

Fries' ('25) experiments with children were carried out from December to March, since the ultra-violet from the sun is not strong enough during these months to confuse the results. Three children were kept on a controlled diet for one month, during which time their basal metabolism was determined three times a week. They were then rayed with a Hanovian mercury arc under various conditions, and the basal metabolism again determined three times a week. Each determination followed the raying by 20 to 70 hours. One patient was rayed 20 times in 24 days, one at irregular intervals, and one 7 times in 16 days. The exposures were at 24 inches at first, then lowered to 16, and began at 3 minutes and were gradually increased to 15. All three subjects became pigmented. Two other children not under controlled conditions were rayed. None of these 5 patients showed any increased metabolism. In two children rayed in the month of April to May, the metabolism rose after the third and fifth treatment and then fell 13 per cent and 11 per cent below normal. The ultra-violet

from the sun may have confused these results. The work of Fries shows that any prolonged rise in metabolism is not usual, and that although individual variation may influence the results, certainly the clinical improvement in malnourished children as a result of raying does not depend on an increase in metabolism.

Fries published later ('27) on the same problem and again found that the metabolism of rayed children did not vary more than 10 per cent. This is an insignificant variation in view of the inaccuracies of determining basal metabolism. In the same year, Fries and Topper ('27) extended the study. The patients were rayed 3 times each week at 20 inches with a mercury arc. The first exposure was 3 to 5 minutes, on the chest and back, and each succeeding exposure was increased 2 to 4 minutes. All patients developed good pigmentation. In the previous experiments, the metabolism determinations were made 20 to 70 hours after the irradiation, but in order not to lose immediate effects the determinations were now made before and after each treatment and also 2 and 6 hours later. There was no change in metabolism, pulse rate, or blood pressure, that exceeded experimental error.

A more extended study was made by Mason and Mason ('26) who rayed 10 patients with the unscreened mercury arc. Eight showed a notable lowering of heat production and a lower pulse rate. In no instance was an increased metabolism observed. The subjects showing a depressed metabolism were referable to two groups: (1) normal patients who pigmented well; (2) patients having an increased amount of circulating bilirubin.

The 5 individuals of the first group showed a lowered metabolism of 13.8, 15.6, 14.5, 7.8, and 10.5 per cent, which was proportional to the degree of pigmentation. Sometimes this lowered rate persisted as long as 23 days after the raying ceased. The 3 patients constituting the second group showed inhibitions of 24.1, 24.5, and 8.0 per cent. The interesting theoretical significance of these results are discussed under the heading, "Theoretical considerations."

Eichelberger ('26) rayed 9 women and 2 men in order to de-

termine whether creatinine was connected with respiratory metabolism. The fact that changes in creatinine excretion had no effect on metabolism convinced this author that they were unrelated.

2. Irradiation as a climatic factor.—Hasselbalch and Lindhard ('11) reported that with increasing altitude there is an increasing sensitivity of the respiratory center. This effect was attributed to the increased ultra-violet reaching the subjects from the rays of the sun. During the first day at a high altitude, there is an abnormally great sensitivity of the respiratory center, but physiological adaptation soon allowed a recovery. The final condition was a subnormal sensitivity. These authors believe that the lowered frequency and deepened amplitude of inhalation were due to the reduced tonus of the vessels in the skin.

A lengthy report, which unfortunately was based on scanty material, was published by Durig and Zuntz ('12). At a higher altitude the respiratory quotient remained constant, although the alveolar carbon dioxide and oxygen tension decreased proportionally to the altitude. The body temperature was not affected. An increased frequency of breathing was shown by 2 subjects, while 1 showed an increase. The amplitude of inhalation also was decreased in 2 subjects, but increased in 1. These results are obviously unconclusive. In the same year, Durig, Schrötter, and Zuntz (12) attempted to correlate high altitude effects with those produced by artificial raying. The result of raying depended on the individual and on the intensity of exposure. The alveolar carbon dioxide was reduced, and many subjects showed a decreased frequency but a greater amplitude of inhalation. It was concluded that climatic effects had no correlation with those of raying.

Kestner and his co-workers have maintained in a series of papers that the short-wave irradiation in high-altitude sunlight stimulated human respiratory metabolism. Kestner, Peemöller and Schadow ('27) reported the results of 5 men ascending a mountain, each person making metabolism tests on himself. The locality was chosen so as to obtain the most in-

tense solar rays possible, but at an altitude of less than 3000 m. so that the oxygen pressure would not be a disturbing factor. The intensity of the radiation was measured by a cadmium cell and an electrometer. This cell almost exclusively reacts to wave lengths less than 3200 Å. Dorno had shown that this length is the most physiologically active on man. This climatic study showed that this spectrum region stimulated respiration and gas exchange. Through over-heating the metabolism was greatly reduced. Wave lengths less than 3200 Å were called by these authors the "Ra" rays. This is an unfortunate term, since it might reasonably be confused with radium emanations if the student were not familiar with the details of the experimental procedure.

During the same year, Kestner and Schadow ('27) reported a similar study carried out at the Jungfrau Pass. The rays shorter than 3200 Å again stimulated the oxygen use. These authors state that the diminished oxygen pressure at this altitude caused a deepened amplitude of inhalation. The "Ra" rays appeared to penetrate relatively thick masses of clouds, and the reflection from the snow was also found very important.

Crofts ('28b) rayed 2 subjects with a "Uviarc" which gave 6.6 lithopone units of ultra-violet in 8 minutes at 18 inches. The dosages were as follows: 5 L. U., 6½ L. U., a total of 20 L. U. distributed over a series of rayings, incrementally repeated rayings giving a total of 17½ L. U., 3 large doses giving a total of 19.8 L. U., incrementally repeated rayings giving a total of 44.9 L. U. distributed over a period of 6 days. Even though these dosages were in excess of usual clinical treatments, there was no appreciable effect on metabolism.

A very excellent review by Lippman ('28) contains the generalization that ultra-violet in low erythema doses increases the oxygen use during the period of exposure 5 to 10 per cent. After 30 minutes, the oxygen consumption falls, often below normal.

A report based on copious clinical material by Lippmann and Völker ('28) is one of the most important contributions in this field. Dosages varied between 1.5 and 6.5 erythema units.

During the actual raying there was an increase in metabolism of 10 to 18 per cent, this stimulation not depending on the size of dose nor upon the intensity of the developing erythema. The fact that mere uncovering of the patients produces some increased metabolism lessens the significance of the results. The respiratory quotient showed a slight lowering, perhaps due to oxidation of the non-carbohydrate substances which Pincussen considers to be proteins. These authors believe that the variations which they obtained in the respiratory quotient were too small and variable to be significant. The volume and frequency of breathing also remained unchanged. There was no time effect during the 24 hours of observation after the raying. There was no significant change in nitrogen metabolism, and the slight decrease in the blood sugar was within the limit of usual variations, being from .32 to .27, .30 to .25, and .24 to .20.

Lehmann and Szakáll ('32a) reported that raying laborers with a mercury arc at weekly intervals lowered their basal metabolism 10 to 15 per cent and increased the capacity for work up to 60 per cent. There was a decrease in the oxygen debt. These effects were thought to be similar to those of muscular training. Another experiment ('32b) reported that when the same subjects were rayed, but with a screen interposed which eliminated the ultra-violet without their knowledge, the increase in work capacity did not take place. Rubow and Sonne ('12) had reported previously that patients suffering with heart diseases reacted favorably to irradiation and that their capacity for muscular work increased.

In a more complete study, Lehmann ('33) rayed a patient for 5 minutes and then measured the heat production hourly for 22 hours. There was a small increase until 7 hours after raying, at which time erythema developed. From this point onward there was a progressive decrease, although sometimes the value was still above normal for several days. In another experiment, weekly averages were obtained for 17 weeks. During the fourth week, the ultra-violet dosages were 3 minutes, and in succeeding weeks they were increased to 6, 10, 15, 20, 30,

and 40 minutes. There was no raying during the remaining 5 weeks. The results are very definite, and the curves representing them are among the best that have appeared. The elimination of carbon dioxide showed no important change, while the oxygen use decreased continuously until the rayings were stopped and then showed a progressive return to the normal during the succeeding 5 weeks. The curve representing heat production parallels very closely the above oxygen changes. The oxygen debt also was parallel to that of heat production.

D. THEORETICAL CONSIDERATIONS

It is difficult to come to positive conclusions concerning the relation of animal metabolism to ultra-violet light. At first glance the experiments of Merker and his co-workers on lower forms show that a 1000-watt filament lamp increases the oxygen uptake, but that a mercury arc always decreases it. However, the presence of eosin confuses any determination of what wave lengths are effective, and, as he himself admits, the increased movement of the rayed organisms may have caused the increased oxygen uptake. Also, the method by which he determined oxygen use is open to criticism, and the curves which he published are extremely unsteady. However, if we give full credence to Merker's results, raying eosin-treated organisms with a 1000-watt light caused an increase of oxygen uptake which resembled that produced by increased temperature. These experiments contribute but little to an ultimate theory of ultra-violet physiology.

The fact that Sonne used pupae, which showed no movement when rayed, and that he used a mercury arc, a much more potent source of ultra-violet, makes his experiments of more significance. He obtained an important increase in oxygen use. Although his organisms were injured, making it impossible to draw conclusions concerning the effects of ultra-violet itself, yet this author's work is the best evidence available that lower forms are stimulated in their oxygen uptake by ultra-violet light.

The work on vertebrates may be summarized with greater assurance. In the absence of factors depending on pigmentation, it is probable that such sensitive organisms as canaries, and, to a lesser extent, white mice, etc., may show increased metabolism. But even here the results are irregular. It is to be noted emphatically that the dosages given in such experiments are greatly in excess of any that might be given therapeutically to human beings, and any suggestion that ultra-violet light owes part of its clinical effect to increased respirational metabolism is without foundation.

The above statement is made with full knowledge of the very positive opinion of Warnshius ('33). This author states that ultra-violet irradiation of human patients "is the most valuable and perhaps the most positive means for the improvement of oxidative processes." This paper contains a large amount of loosely organized theory purporting to show that ultra-violet light has a greater anti-rachitic power than vitamin D, which he attributed to the improved oxidative power of the blood enzymes. He further states that vitamin D is probably only another oxidative enzyme or a precursor or an intermediary of other oxidases. Ultra-violet acts, then, by activating the oxidizing enzymes and this affects the mineral metabolism. This theory cannot be substantiated by data in the literature.

Ultra-violet experiments with human beings have not always been above criticism. Scanty clinical material, poorly controlled conditions, and faulty technique have thrown an undeserved appearance of futility in this field of research. After evaluating the various published results on the basis of the conditions under which they were obtained it is fairly certain that ultra-violet irradiation gives no stimulation of respiratory metabolism. If the effects of irradiation are influenced by pigments, such as melanin, bilirubin, etc., there is a decrease in oxygen use because the pigments degrade the incident irradiation into the longer wave lengths. The total effect is, then, the same as the addition of heat to the body, and consequently there is need of less intracellular oxidation. This viewpoint might be conclusively checked by raying negro and

white patients. The difference in pigmentation is here so great that there should be a marked drop in oxygen use by the colored patients long before the pigments could develop in the white patients, and the usual unavoidable variations should become insignificant.

Merker ('25c), in a long review of the biological effects of fluorescent dyes, suggests that they may act either as oxygen carriers, or under other conditions they may destroy cellular oxidizing mechanism. These suggestions are worthy of consideration, but it is possible that fluorescent substances inhibit by some physiological effect, depending on their emission spectra.

The mechanism by which photo-sensitizers affect oxidation is still controversial. Gaffron ('33) believes that the oxidizing effect of irradiated dye solutions depends on an activation of the oxidizable substance. An extended study of this problem was carried out by Kautsky, Bruijn, Neuwirth, and Baumeister ('33). They believe that the photodynamic effect of a fluorescing dye is due to a meta-stable condition of oxygen. The oxygen absorbs energy from the fluorescing dye molecule and thereby causes fluorescence to disappear. Since the meta-stable oxygen has a finite time existence it may diffuse away from the point of activation to an oxidizable body which is itself not irradiated. This mechanism was convincingly demonstrated by irradiating tryptoflavine and observing the resultant oxidation of leuco triphenylmethane and also leuco malachite green. These reduced dyes were maintained in silica jell some distance from the point of irradiation.

The statement of Wickwire and Burge ('27) that ultra-violet inhibits the oxygen use of Paramecia by destroying the activity of insulin is suggestive, but it seems a far cry to assume that these organisms are so similar to higher forms as to possess an identical mechanism of sugar utilization.

The reports covering the stimulation of metabolism by solar rays and under various climatic conditions are subject to too much criticism of technique and are based on insufficient material to be of theoretical importance. This is also the attitude of Flickinger ('26).

III. Metabolism of Plants

A. FUNGI

1. Bacteria.—Schnitzler and Henri ('09) were the first to observe the effect of radiations from a mercury arc on bacterial metabolism. They studied acetic acid fermentation of various wines, red Algerian, white Algerian, and a mixture consisting of 100 parts of wine, 200 parts of water, and 4 parts of glacial acetic acid. The solutions were irradiated at a distance of 15 cm. in 50-cc. samples, 1 cm. deep, and in 200-cc. samples, 4 cm. deep. An exposure of 30 minutes completely stopped acetic acid fermentation, while shorter exposures were inhibitory to a less degree. Special tests indicated that the ozone formed by the ultra-violet light was without effect, and that wave lengths shorter than 3021Å constituted the effective spectrum region. It is particularly significant, as will be pointed out later, that if the wine were rayed in the absence of oxygen the ultra-violet was without effect.

This paper was followed by a more complete report the following year ('10). The irradiation was carried out as before, and the cultures watched for 3 to 9 days in a water bath maintained at 25° C. The experimental solution consisted of 100 parts of red wine, 200 parts of water, and 4 parts of glacial acetic acid. Irradiation for 30 minutes decreased the amount of acetic acid from the control value of 5.90 to 5.05 per cent. Shorter dosages were proportionally less inhibitory. The fact that even 40-minute rayings were without effect in the absence of oxygen emphasizes their previously published results. Traces of hydrogen peroxide gave not only similar inhibitory results but also produced the same color changes in the wine as did irradiation. Hence it was concluded that the toxic effect of ultra-violet irradition by wave lengths shorter than 3021 Å depends on the formation of hydrogen peroxide in the presence of atmospheric oxygen.

The actual possibility of hydrogen peroxide developing in irradiated water solutions will be more fully discussed in the section on peroxidase.

The first data concerning the effects of ultra-violet light on

the respiration of bacteria in pure culture were reported by Cook and Stephenson ('28). They studied primarily the relation of viability of the bacterial cell to its aerobic oxidation of glucose and certain of its fermentation products, and irradiation with ultra-violet was only incidentally introduced as a means of reducing viability. They grew *Bacillus coli* on tryptic broth agar to which .5 per cent sodium lactate had been added. After 24 hours, the cells were suspended in phosphate buffer at pH = 7.4, centrifuged, then washed twice in Ringer's solution, and finally suspended in 100 cc. of Ringer's solution, and aerated for 15 minutes in order to obtain an even suspension. This stock suspension was diluted with an equal volume of Ringer's solution, and 1 cc. was used for each determination. Glucose, 1/200 M, was used as the control culture. The substrates, lactate and pyruvate, were studied in 1/100 M concentration, and acetate, ethyl alcohol, acetaldehyde, formate, oxalate, and glycollate were also studied. In order to decrease the viability of the cells, they were rayed in quartz test-tubes at a distance of 6 inches from a mercury arc for 20 and 30 minutes. Super heating was avoided by protecting the culture tube by another and larger test-tube. The suspensions rayed for 20 minutes showed 2.74 viable cells in each 1000; those rayed 30 minutes showed 2 viable cells in each 1000.

The effects of ultra-violet on the respiration process, aside from the mere killing of most of the cells, can only be inferred. At first, there was a slight decrease of oxygen use, but later there was no significant difference. This preliminary period of slight inhibition was attributed to an injury to the respiratory enzymes, although no actual tests were made. Since a reduction of the number of cells by dilution gave a rate of oxygen use proportional to the number of cells, and since a reduction of living cells by irradiation did not materially affect the respiration, these authors concluded that living and dead cells oxidize alike.

The oxygen uptake was measured by differential manometers of the Haldane-Barcroft type. It was assumed that if the control culture were placed in the vessel on one arm and the

experimental culture in the vessel on the other arm, the movement of the manometer liquid would indicate the difference in rates. If all the forces acting on each limb of the manometer were perfectly symmetrical, then the above assumption would be valid. This can rarely be the case in actual experimental procedure, as can easily be determined by placing duplicate aliquots in each vessel. Experiments by Wynd have shown the unsuitability of this method, and errors from this cause alone might easily be of such magnitude as to account for any of the differences between the normal and rayed cells in the experiments reported above.

Lohmann's ('34) study of *Bacillus coli,* tubercle bacilli, and *Streptococcus* is the only one that has been directed specifically to determining the effect of ultra-violet on the respiration of bacteria. *Bacillus coli* was grown in a buffered Ringer solution which contained sugar. This medium allowed no reproduction, and the cultures exhibited a constant rate of oxygen uptake as determined by the Warburg method. A bouillon medium which allowed reproduction was also used. Tubercle bacilli were cultured on Lockemann's medium. The most complete data reported in this paper concerned *Streptococcus.* The culture was diluted with 9 volumes of colorless Ringer solution at pH = 7.4. An exposure in a quartz flask to a mercury arc for 45 minutes lessened the oxygen uptake about 50 per cent, and this rate was maintained for several hours. After 7 or 8 hours new growth caused an increase in oxygen use and in elimination of carbon dioxide. An exposure of 80 minutes stopped growth completely and almost all of the respiration, but new growth began after 12 hours. Rayings as long as 3.5 hours did not completely sterilize the cultures.

Experiments on *B. coli* showed that exposures of 3 minutes resulted in a constant use of oxygen, showing that growth had been stopped although metabolism remained uninjured.

2. Botrytis.—Fazi ('23) reported that fermentation of grapes infected by *Botrytis cinerea* was stimulated by exposure to the mercury arc. Although it cannot be said that this result is not valid, yet, as will be shown later, all of the work reporting

increased fermentation in yeast by ultra-violet is open to serious criticism.

3. Yeast.—The effect of irradiating yeast on respirational activities was first observed by Maurain and Warcollier ('09). They used a mercury arc of the Poulenc type, operating on 110 volts and 2.5 amperes. Sweet cider was rayed in layers 1 mm. deep, at a distance of 5 cm. An exposure of 3 minutes killed fermentation. If the cider were diluted with 20 parts of water, then 2 minutes stopped fermentation. If the layer were reduced to ¼ mm., 2 to 3 minutes were completely inhibitory.

In the following year, these authors ('10) extended their observations to foaming white wine in an effort to determine if irradiation might be used to inhibit the undesirable late stages of fermentation. The wine was in layers 5 mm. deep, and was 4 cm. removed from the mercury arc. Irradiation for 10 seconds completely inhibited fermentation. The greater sensitivity of the white wine was due to its greater transparency for the effective radiations.

The most prolific writer in this field of research is Fazi. His name first appears in the literature in the announcement of a British patent ('14) for carrying out commercial fermentation under the influence of ultra-violet light. He points out that either sunlight or "Uviol" lamps may be used as the source of irradiation. The fermentation vats were to be made of "Uviol" glass, or the lamps immersed directly in the liquids. The announcement of this patent was followed by a long series of papers, all purporting to show great stimulation of fermentation by ultra-violet light. The first paper reported that beer yeast could be rayed profitably even for as long a period as 12 hours, and that it was not injured by 14 hours of exposure. The irradiation was carried out in quartz flasks, 20 cm. from the mercury arc.

Fazi and Fazi ('16) describe experiments showing that *Saccharomyces opunitiae* lives, reproduces, and ferments more actively when stimulated by irradiation. Many hours of exposure were needed to cause injury. Also, the must of India figs forms abundant film on the surface in ordinary fermenta-

tion, but under the influence of ultra-violet there was no trace of film. These authors later ('17) mixed *Saccharomyces cerevisiae* with *S. opunitiae* and found that the rate of fermentation of fig must was doubled by ultra-violet light. The change of sugar to alcohol and carbon dioxide was almost complete, while in the unrayed cultures there remained a residue of sugar. Pure cultures of *S. cerevisiae* also were rayed in fig must, and at the end of the experiment the samples contained less acid, about 10 per cent more alcohol, and a smaller residue of sugar.

Fazi ('21) published new data in order to contradict a paper by Feuer and Tanner ('20), who had stated that their yeast could not endure more than a 1-minute exposure. Fazi not only states that this result is opposed to his own, but also to the observation of many others. He mentions particularly Henri and Stodel ('09), Cernovodeanu and Henri ('10), Vallet ('10), Lombard ('10), Van Aubel ('09). Fazi claims to have done much work on bacteria and on yeast, although there is no extensive publication of data. In the above paper he described experiments in which brewer's yeast was rayed for 12 hours with a 1200-candle power lamp at 20 cm. The yeast was not injured and showed an increased fermentation, but all the bacteria in the suspension were destroyed. This method of clearing yeast from bacteria was instituted in 1918 by the Peroni Brewery, in Rome.

The significance of Fazi's paper was completely nullified by the reply of Feuer and Tanner ('21) in which they state that Fazi misquoted them in saying that the yeast died with 1 minute of raying, since they actually had said that some samples endured exposure for 7 minutes. It is significantly pointed out that the suspensions were very dilute, consisting of a single loopful of inoculum in 9 cc. of water, and then rayed in thin layers at a distance of 25 cm. It is a surprising fact that none of the 5 papers cited above by Fazi in support of his results mentions yeast in any connection whatever!

Fazi and Fazi ('22), in support of their previous contentions, say that tests on an industrial scale confirm their results in the

laboratory, that yeast previously treated by ultra-violet exhibited stimulation of fermentation activities. The possibility of commercial applications was pointed out for the third time two years later ('24). This paper claimed that ultra-violet reduced the time for proper fermentation of beer 25 per cent, reduced time of maturation 40 per cent, and that it improved the flavor and storage qualities. The temperature of the fermenting liquid might be held as low as 4° to 6° R., and the fermentation was still greater than at the usual warmer temperatures.

The last paper by Fazi ('27) describes the effect of raying glucose solutions before inoculation by *Saccharomyces cerevisiae*. Although the yeast itself was not rayed, it showed an increased fermentation and produced a smaller amount of acid. He concludes that the preliminary exposure of the glucose solution imparts bactericidal properties, which, however, do not inhibit the yeast. Fewer bacteria were found in solutions which had been rayed.

Lindner ('22) was one of the earliest supporters of Fazi. He used bottom brewer's yeast and found that the velocity of fermentation was increased by ultra-violet to an even greater degree than originally claimed by Fazi for top yeast. For instance, 30 gms. of glucose in 300 cc. of water yielded in 24 hours 119 cc. of carbon dioxide, while a similar solution rayed with a mercury arc gave 2743 cc.! The original and the rayed yeast contained but little glycogen, but significant amounts were present in yeast cells that were allowed to ferment in the absence of ultra-violet. This is consistent with the results on fermentation. A most interesting observation in this paper is that 20 to 30 per cent of the cells in the rayed solutions had died. The present writers' experiments on yeast, which are reported in the third number of this series, show that fermentation is inhibited by ultra-violet even *before* a significant number of the cells show mortality. Minor variations in the substrate were shown by Lindner to be ineffectual in disturbing the results, since the use of wort gave similar effects to that of glucose.

A more scientific approach is that of Gronchi who showed

('32a, b) that the shorter wave lengths were toxic to fermentation of yeast. He reported irradiating *Saccharomyces cerevisiae* for 45 minutes at 40 cm. Wood's light caused an increased production of carbon dioxide which continued some time after the irradiation stopped. The shorter ultra-violet excited fermentation, but this increase was of short duration and stopped when the irradiation did, and from this point onward the elimination of carbon dioxide was diminished.

The next attempt to show a favorable effect by ultra-violet light on yeast fermentation is that of Owen ('33) and his coworkers. He used a National Carbon Company carbon arc, which is rich in the wave lengths 2300 to 3100 Å. Seven grams of yeast cake were added to 100 cc. of 5 per cent glucose and exposed in a shallow pan. Exposures of only 10 or 30 seconds imparted an increased fermentative power. The greatest difference between the control and experimental cultures occurred about 1 hour after irradiation. Owen reported a commercial application of irradiated yeast to bread baking, since 24 per cent less yeast is needed if it be previously rayed 10 seconds. However, it is highly probable that the quantity of yeast ordinarily used by bakers is in excess of that needed, and the fact that 24 per cent less gives proper leavening does not prove any beneficial effect of irradiation.

The most extensive report on stimulatory effects is that of Owen and Mobley ('33). These workers rayed seed yeast in the same type of solution as was to be fermented, the wort itself and colonies on molasses agar. The wort was prepared from Cuban Black Strap molasses to which was added 1 cc. of sulphuric acid and 1 gm. of ammonium-sulphate per liter. Pure cultures of Magne yeast were used. The raying was accomplished by dripping the wort through a funnel at 15 cm. from a carbon arc.

In one experiment type "C" carbons of the National Carbon Company were used, which gave radiations from 2700 to 3000 Å. The inoculated wort was run through the funnel three times before the arc. After 72 hours the efficiency of the alcoholic fermentation was as follows:

Control.....80.62 per cent
Control.....83.20 per cent
Rayed.....83.10 per cent

These results are not impressive evidence for stimulation.

Another experiment was carried out with type "B" carbons, and the fluid passed 5 times through the funnel. After 72 hours the results were:

Control.....88.9 per cent
Rayed.....90.8 per cent

A third experiment differed from the above in that the suspension was passed 6 times through the funnel. After 48 hours the efficiency of the alcoholic fermentation was:

Control.....83.3 per cent
Rayed.....85.2 per cent

It was further reported that raying the yeast on agar plates and then transferring it to the wort were almost as stimulative as raying the inoculated wort. Irradiation of the media was also held to be beneficial. "B" carbons were used, and the distance is not given. The results were:

Control.....80.3 per cent
Control.....82.8 per cent
Colonies rayed.....83.4 per cent
Colonies rayed.....80.3 per cent
Seed rayed in wort....87.5 per cent
Seed rayed in wort....84.0 per cent

The scant data and the obvious variation in the duplicates are not convincing evidence for stimulation. These authors concluded with the statement that irradiation by the carbon arc was beneficial because of the development of some "growth-promoting" substance which stimulated the fermentation of sugar. It is obvious that a "growth-promoting" substance is something that promotes *growth* and not necessarily the fermentation of sugar. This improper terminology is more plainly demonstrated by the values given for the actual num-

ber of cells per cc. as related to the period of irradiation. These values after 32 hours were:

Control	168,000,000
Rayed 1 minute	116,000,000
Rayed 2 minutes	182,000,000
Rayed 3 minutes	48,000,000
Rayed 4 minutes	82,000,000
Rayed 5 minutes	12,000,000
Rayed 6 minutes	16,000,000

In spite of the erratic values, which vary from about 30 to over 100 per cent from a smooth curve, there is evidently no "growth-promoting" substance acting as a result of irradiation.

Surányi and Vermes ('29), employing the Warburg technique, were the first to observe the effect of irradiation on the use of oxygen by yeast. The nutrient medium was the Tyrade solution at pH = 7.2. Exposures to ultra-violet light gave an increase in oxygen use of about 50 per cent. Cyanide inhibited the increased fraction of the total as well as normal respiration, and this effect was reversible. Avian erythrocytes gave similar results.

The evidence of the inhibitory influence of ultra-violet on fermentation is more convincing. Söhngen and Coolhaas ('23) were the first to differentiate between decrease of reproduction and of fermentative power. They observed that neither fermentation nor reproduction was affected unless the distance from the lamp was great, the volume of solution large, and the time of exposure short.

Tanner and Ryder ('23) extended their earlier work in view of the objections raised by Fazi. They rayed 20 different species of yeast with a Cooper-Hewitt lamp operating on 110 volts and 4 amperes, at 20 cm. from the cultures. One loopful of each was inoculated in 100 cc. of sterile water, and 1 cc. of this suspension was added to 99 cc. of sterile water. The final suspension was rayed in layers 8 mm. deep in Petri dishes, after which 1 cc. was plated on acid Sabouraud's agar. Ex-

posures of 1.5 to 20 minutes were required to kill the various species. The loss in weight from 30-day cultures of *Saccharomyces ellipsoideus* showed that 1- to 10-minute exposures had no effect on fermentation, but that longer exposures inhibited proportionally. Since bacilli were killed under similar conditions in 1.5 to 6 minutes, it was concluded that the size of the cell determines its resistance to ultra-violet.

Abderhalden ('27) raised the next dissenting voice. He rayed fresh yeast, dry yeast, macerated yeast, and press juice with a mercury arc and with a magnesium light. He added both rayed and unrayed egosterin and cholesterin, and under no condition did he find stimulation of fermentation. He concluded that fats are not concerned in yeast fermentation, and from his data it may be inferred also that ultra-violet itself was not stimulatory.

Tanner and Byerley ('34) reported the inhibitory effect of ultra-violet on *Saccharomyces cerevisiae* and on Fleischmann's commercial pressed yeast cake. They rayed glucose-broth cultures, aqueous suspensions before seeding, fermenting cultures and glucose-broth before its inoculation, with a Cooper-Hewitt mercury arc at a distance of 25 cm.

Oster ('34), in the most careful study yet made on the subject, reported the effect on respiration in terms of oxygen use. He used monochromatic light from the mercury arc, including all those lines which are of sufficient intensity to be practically applied. The results were reported in terms of the wave length used, the total energy in terms of ergs per sq. mm. per second. The oxygen use was observed by the Warburg technique. The respiration curves were run for 2½ hours. At the end of the experiment, the suspension was removed and plated on agar to discover any morphological changes induced by the raying. The conclusions were that there was no stimulation of growth and no change in the oxygen use until the cells were injured as shown by morphological studies when there was only a decrease. About 22 per cent decrease in oxygen uptake occurred when the total energy exceeded 81.6×10^4 ergs per sq. mm. per second, this value being obtained by exposure to the line 2536 Å

for 60 minutes. The region 2482 Å to 3132 Å showed about the same degree of inhibition, although the total energy was 408×10^4 ergs per sq. mm. per second. The effect is not one of total energy alone but also one of individual wave lengths, since it is proportional to total energy for any one wave length, but different total energies were needed for the different wave lengths to produce a given effect. This paper is one of fundamental importance and contains too much material to be reviewed completely here.

B. GREEN PLANTS

The only study on the effects of ultra-violet light on the respiration of higher plants is that of Masure ('32). He used a Cooper-Hewitt mercury arc operating on 110 volts and 4 amperes, and a Corning G586AW "Ultraglass" filter, 9 mm. thick, and passing wave lengths from 3334 Å to 3690 Å. Because of the characteristics of the mercury arc, this filter showed a peak of transmission in the region 3650 Å to 3663 Å. The mercury arc triplet at 3663 Å, 3665 Å and 3650 Å constituted about ¼ of the total radiant energy passed. Since the line at 3650Å was about ½ of the total intensity of the triplet, the light was practically monochromatic at 3650 Å. This line has been shown to be within the transmission region of certain seed coats.

Three varieties of pea seeds were rayed in Petri dishes for periods of 15, 30, 60, 120, and 265 minutes, and planted immediately after. The hypocotyls were measured after 4 days to determine the effect of raying on growth. Respiration was observed in etiolated seedlings rayed at 27 cm. through the G586AW filter. The flask was in a water bath, which caused the elimination of the longer infra-red. Since window glass transmits down to 3200 Å, the thin glass of the flask probably passed most of the band at 3650 Å. The seedlings were rayed 62 minutes, then after 50 minutes they were rayed a second period of 49 minutes. The technique of measuring the oxygen uptake was not so refined as might be wished, but it appears from these experiments that the absorption of oxygen was temporarily increased by irradiation.

C. THEORETICAL CONSIDERATIONS

The work of Cook and Stephenson might be interpreted as indicating that *Bacillus coli* was unaffected, but, as pointed out above, the balancing of one limb of the differential manometer against the other introduces errors that might conceal small differences. It should be noted, however, that this error need not be present if the two vessels were identical *in every way*. This could be determined experimentally by putting duplicate aliquots in both vessels; and should the manometer liquid remain at a constant level, then the method of Cook and Stephenson would be sound. But these authors specifically state that they "assumed" this condition. The present writers have never been able to obtain symmetric behavior of pairs of manometer vessels. The work of Lohmann shows only inhibitory effects.

The work on acetic fermentation is even more difficult to interpret, since no data were given that would make possible the necessary distinction between inhibiting the fermentation itself and the actual killing of the fermenting organisms. It is significant that acetic acid fermentation was not inhibited if the irradiation occurred in the absence of oxygen. This indicates that the inhibiting action does not depend on the specific effect of the ultra-violet in destroying the oxidizing mechanisms, but rather on some toxic oxidation that ultra-violet induced by the presence of oxygen.

The earlier work on yeast fermentation is open to the same objection, since no distinction was made between fermentative activity and the killing of the cells. The evidence for the stimulation of yeast fermentation centers around the work of Fazi and of Owen and their co-workers. When we note that Fazi's name appears twice in the announcements of commercial patents, and three times in articles emphasizing the desirability of commercial application, the actual amount of new scientific evidence advanced to support his belief is small. The work of Owen and co-workers is to be criticized even more severely. Their actual experimental results, which have been partly quoted above, are sufficient indication that no significant differences were found.

The favorable effects of irradiating the nutritional medium reported by both Fazi and by Owen are worthy of further study. Woodrow, Bailey, and Fulmer ('27) found that the irradiation of sucrose, glucose, calcium gluconate, and glycerol imparted toxic properties to yeast. Euler and Lindberg ('12) found that rayed glucose solutions develop a gas mixture containing about 15 per cent carbon dioxide, 40 per cent carbon monoxide, and 40 per cent hydrogen. They also showed that ultra-violet light anaerobically split lactic acid in water solutions into alcohol and carbon dioxide. These reactions are all purely photochemical, and such effects must be considered in interpreting results.

The work of Bierry ('26) is particularly interesting because he also found that ultra-violet could act on carbohydrates similarly to certain enzymes, making possible an "ultra-violet fermentation." He found that d-fructose, and to a less extent d-glucose, in the presence of air is degraded by ultra-violet to formic aldehyde and carbon dioxide. Sucrose is hydrolyzed by the irradiation and then degraded to carbon dioxide and formic aldehyde.

The evidence against the stimulation of yeast fermentation is based on more stable foundations. The present writers report their results in the third paper of the series.

There is not yet sufficient evidence in the literature concerning the effect of radiation on the higher plants to form definite conclusions. The present writers report in the second number of this series their work on tomato and bean plants.

IV. Oxidizing Enzymes

A. CATALASE

1. In vivo studies. a. Animals.—Ostwald ('08) rayed larvae of *Porthesia chrysorrhoea* with sunlight and found that both violet and white light reduced the amount of catalase activity below that found in larvae treated with yellow light or with darkness. The amount of ultra-violet light reaching the organisms in these experiments is very uncertain.

The first *in vivo* study of the effects of relatively intense

ultra-violet light on animal catalase appeared in 1926. Pincussen, in a series of papers on the changes in the enzymatic content of the blood, points to the probability that ultra-violet affects the organism by increasing oxidation. This assumption was based on previously published data showing that ultra-violet exhibited an oxidative effect when studied *in vitro*. Recent developments, particularly in the chemistry of the enzymes, show that *in vitro* studies are not necessarily applicable to the interpretation of *in vivo* phenomena.

Pincussen approached the problem by observing the catalase activity of the blood of rabbits after they had been exposed to bright sunshine from 10 o'clock a. m. to 4 o'clock p. m., and also after being irradiated by a mercury arc. Animals in the sunlight always showed a lower reaction constant of the catalase than those kept in darkness. The magnitude of this decrease was not changed materially when 2 cc. of 2 per cent erythrosin were injected subcutaneously. Neither were 10 cc. of 2 per cent potassium iodide administered orally effective in altering the effect of sunlight. Rabbits irradiated for 12 minutes by a mercury arc at a distance of 100 cm. showed a lowering of the reaction constant from .0621 to 0.410. That this decrease was not a transitory effect was shown by raying rabbits 12 minutes at a distance of 90 cm., and withdrawing blood samples periodically until 120 minutes had elapsed. The reaction constant showed a progressive decline as long as the observations were continued.

The blood catalase of animals rayed by the mercury arc was markedly more decreased in the presence of potassium iodide and eosin than in those treated with sunlight. This was explained by assuming that the eosin was not acting as a photosensitizer for catalase, but by increasing the amount of free iodine splitting from potassium iodide. In these experiments there was also a progressive decrease during the 2 hours of observation after raying.

Somewhat different results were obtained by Castagna ('27a). After keeping white mice in the dark, he rayed them with the mercury arc, using both the visible rays plus the ultra-

violet and also the ultra-violet alone from 3900 Å to 3190 Å. The catalase of the blood was determined at intervals of one-half hour, and it was found to increase during the first 3 hours of raying when the open arc was used and then to drop to normal after several hours. When the wave lengths 3900 to 3190 Å were used alone, there was no significant change. This author's conclusion, that visible light increased the enzyme or protected it from destruction by some natural process, and that irradiations shorter than 3190 Å were destructive, does not adequately explain these results. It should be noted that these dosages are very much greater than those used by Pincussen.

It will be remembered that Harris ('25a), in his study of the effect of light on the carbon-dioxide elimination of rats, found that the open mercury arc had no effect, while the ultra-violet of 2910 Å to 4360 Å gave a stimulation. He concluded therefore that visible rays nullified the ultra-violet stimulation by physiological antagonism.

Koldaev and Al'Tshulla ('30a) published data that is compatible with those of Pincussen. They rayed 20 rabbits with a mercury arc and observed the changes in catalase, lipase, and amylase in the blood. Single rayings gave a temporary decrease in catalase, while successive doses produced a progressive decrease. The above data appeared in Russian and was apparently republished the same year in German ('30b).

Pincussen and Tanino ('31) rayed white rats, which had been starved for 16 hours, with a mercury arc operating on 220 volts at a distance of 50 cm. The animals were then killed and 10 cc. of the diluted tissue extract were added to 10 cc. of 1 per cent hydrogen peroxide. The mixture was buffered at pH = 6.8 by phosphate. The results were as follows:

CUBIC CENTIMETERS OF OXYGEN EVOLVED IN 5 MINUTES

	1 cc. blood	1 gm. liver	1 gm. spleen	1 gm. heart
Control	1427	7129	606	178
Rayed 30 minutes	1534	5659	722	182
Rayed 60 minutes	1454	5173	665	172
Rayed 90 minutes	1648	7104	700	157

The significance of these data will be discussed under the topic "Theoretical considerations."

1. In vivo studies. b. Plants.—In vivo studies on plants concerning the effect of irradiation on the catalase are less numerous than those on animals. The effect of sunlight on yeast catalase was observed by Euler and Laurin ('18). They mixed 15 cc. of a suspension of *Saccharomyces thermantitonum* containing .0885 gms. of fresh yeast with 100 cc. of phosphate buffer at pH = 6.1 and with 50 cc. of .01 N hydrogen peroxide. Exposure of the yeast to sunlight uniformly lessened catalase activity.

Novikov and Herber ('33) soaked the seeds of the tau-sagyz rubber plant in water for 18 hours and then irradiated them by a mercury arc. The seeds were kept at 25° C. for 2 days and the catalase of the germinated seedlings then determined. The radiation gave an abrupt rise in catalase.

The paper by Fuller ('32) is the only publication to date reporting the effects of raying on the catalase activity of growing plants. Tomato plants and Red Kidney field beans were grown in individual 4-inch pots to a height of about 20 cm. They were then rayed 3 minutes daily for 7 days at a distance of 50 cm. from the arc, the rays being filtered through a layer of 1.5 cm. of water in a quartz cell. Serious injury was produced, as shown by the bronzing and curling of the leaves. At the end of the 7-day period, catalase was determined by a modification of the Appleman method. The averages showed that the catalase activity of the beans had been stimulated 43.2 per cent and that of the tomatoes 49.7 per cent.

2. In vitro studies.—Catalase was studied in its relation to ultra-violet light by *in vitro* studies much earlier than it was approached by *in vivo* methods. It is surprising that the first of these reports was also the first one concerning catalase and ultra-violet in any connection and was carried out by monochromatic radiation. Hertel ('04), in a preliminary study of several sources of ultra-violet, noted the effect of the magnesium line 280 mμ on peroxidase and catalase. In the cata-

lase experiments, he rayed fragments of fungus mycelium, milk, and fibrin. When a drop of hydrogen peroxide was then added, the evolution of oxygen bubbles under the microscope seemed to show that the irradiation had been without effect. But when these substances were rayed in the droplets of peroxide solution, there was a great increase in the evolution of oxygen over that in the unrayed droplets. Since drops of pure peroxide solution foamed when rayed, Hertel correctly assumed that his experiment did not demonstrate increased catalase activity. It is regrettable that his admirably arranged monochromatic irradiation could lead to no significant results because of the very poor method of determining the effect of raying. His results have been quoted by several authors as an indication that catalase activity was increased. A reading of the original paper shows this to be in error.

Catalase preparations from *Dytiscus marginalis* were exposed to sunlight by Ostwald ('08). The activity was determined by titrating the hydrogen peroxide that remained in the test preparation after the lapse of a definite period of time. The enzyme from the larvae exposed to sun used up 94.7 per cent of the available hydrogen peroxide, and the control destroyed 96.8 per cent. While these values show a slight injury to the catalase, it is probable that they are insignificant. The present writers have found that the titration of duplicate aliquots of catalase by the method of Ostwald gives very erratic results unless the amount of peroxide added as a substrate is sufficiently great to insure at least 15 per cent of the original amount still present at the time of titration.

Catalase from rabbit and from human blood was found by Lockemann, Thies, and Wichern ('08) to be greatly injured by 2-hour exposures to strong sunlight. The preparations were exposed while they were reacting with the hydrogen peroxide substrate. Controls containing peroxide alone allowed a correction to be made for the amount of peroxide destroyed by sunlight itself.

The relation of photosensitizers to catalase was studied particularly by Zeller and Jodlbauer ('08). An exposure of 25

minutes to sunlight filtered through water injured the catalase activity 68 per cent if oxygen were present. In the absence of oxygen there was only 10 per cent injury. Exposure to a mercury arc for 30 minutes showed 29 per cent injury if oxygen were present and 25 per cent if absent. These figures show that oxygen is necessary for the injurious action of the visible light and is unnecessary for the ultra-violet. Increasing alkalinity was found to increase the injurious effects of the sunlight, while corresponding increases of acidity up to 1/1500 N sulphuric acid were without effect. The sunlight was increased in its destructive action by 1/5000 N eosin only if the light were passed through a glass plate to remove the short ultra-violet. The eosin had no influence on the irradiation from the mercury arc unless a glass plate was interposed as a filter. Essentially the same results were obtained by the use of sodium-dichloranthracene, methylene-blue, and Bengal-rose.

The injurious waves of the emission spectrum of the fluorescent substances might depend on the nature of the incident light, but why this should be prevented by the mere additional presence of ultra-violet is difficult to explain. It would seem that the destruction of both types of rays should be additive. If, however, the above results are authentic they furnish an interesting instance of antagonism. It is not possible to surmise whether this antagonism is one of physical interference or of physiological reaction.

Some phases of the work of Zeller and Jodlbauer were criticized by Battelli and Stern ('10). They found that the destruction of catalase by the visible sun rays proceeded equally fast in the presence or absence of oxygen. Although the sun can rapidly and completely destroy catalase, a protective action is exerted by traces of alcohol, aldehyde, and certain other chemical agents.

Agulhon ('11) studied the behavior of a number of enzymes to the radiation from a Heraeus mercury arc operating on 110 volts and 2 to 3 amperes. The enzyme preparations were rayed in layers about 1 mm. deep in quartz tubes at a distance of 15 to 20 cm. The activity of catalase obtained from pork fat was

reduced to 83 per cent of the control by an exposure of 1 hour, to 39 per cent in 3 hours, and to 8 per cent in 6.5 hours.

A more complete report was published by Agulhon ('12) the following year. He found, contrary to Battelli and Stern, that catalase from pork liver was destroyed in the absence or the presence of oxygen by visible light, although to a less extent in vacuum, and, in contrast with Zeller and Jodlbauer, he found that the destruction by ultra-violet was notably lessened in vacuum. These inconsistencies were explained by the probable traces of oxygen in evacuated tubes which might be ample to effect the destructive oxidation of the comparatively minute traces of actual enzyme material.

Waentig and Steche ('12) rayed with a mercury arc catalase prepared from fat, liver, blood serum, alcohol, precipitate of blood, larvae of *Sphinx ligustris*, pupae of *S. ligustris*, and from fungi. In all cases the catalase activity was lowered. The injury was small when the raying took place in glass tubes. In some instances, nitrogen was bubbled through the solution for a long time to eliminate the oxygen, but injury was not prevented. It was concluded that the injury was not oxidative, but probably an effect on the solubility of the enzyme, since more or less precipitate accompanied the destruction of the enzyme. Since alkaline solutions increased the injury while acid did not, these authors concluded that probably all catalase reacted the same. Zeller and Jodlbauer found similar results with fat and blood catalase.

The remarkable stability of the catalase activity of red blood corpuscles was demonstrated by Peemöller and Franke ('26). They suspended washed human blood corpuscles in a .9 per cent sodium chloride solution. One cc. of the suspension was added to 10 cc. of 3 per cent hydrogen peroxide, and aliquots of 1.5 to 2.0 cc. were rayed in thin layers at a distance of 50 cm. Under these conditions an 8-minute exposure gave 1 HED (Hauterythemdosis) unit. Exposures up to 10 HED units (80 minutes) did not produce any significant injury.

It is important to distinguish in studies on the catalase activity of blood between the activity of hemoglobin and that of the blood catalase proper. The great and unexpected stability

of the catalytic action of "Blutkatalase" of Peemöller and Franke is not concerned with the true *enzymatic* activity. This distinction was taken into consideration by Pincussen and Seligsohn ('26). These authors point out that errors due to the activity of hemoglobin can be lessened by proper buffering, since the optimum pH for blood catalase differs from that of hemoglobin.

The experimental preparations of Peemöller and Franke were obtained by adding .02 cc. of blood to 50 cc. of water. Aliquots of 5 cc. of this dilution were mixed with 100-cc. portions of 1/2000 hydrogen peroxide. The reaction was stopped by adding 2 cc. of 20 per cent sulphuric acid. The remaining peroxide was titrated with N/100 potassium permanganate. Aliquots of 25 cc. were removed for titration after 6, 15, and 20 minutes. Irradiation for 20 minutes at a distance of 10 centimeters greatly injured the catalase. This injury was greatest at the pH that is optimum for catalase activity, and was also proportional to the degree of dilution. The presence of potassium iodide and sodium chloride exerted some protective action against destruction of the enzymatic activity by enlarging the colloidal particles sensitive to light.

Sodium chloride was itself injurious in addition to its effect on the light injury. In the presence of this salt, about 50 per cent of the activity was lost on raying for 10 minutes, while in its absence the injury was 25 per cent. The action of potassium iodide is complicated. There is an injury due to the splitting off of free iodine, but in an excess of the salt the protective action of the salt itself exceeds this injurious effect. It is impossible in this instance to clearly distinguish the effect of the salt as a protector, the injurious effect of the halogen ion, and the injurious effect of the liberated iodine. The toxicity of liberated iodine can be demonstrated by raying catalase with alival.

Blood from dogs was exposed in quartz tubes to ultra-violet by Castagna ('27b), and the catalase was progressively inactivated, becoming almost zero after 1½ hours. Sunlight gave a very slight diminution.

Morgulis ('29), working with kidney catalase at various hy-

drogen-ion concentrations, found complete inactivation at all degrees of acidity, but the required time varied. At pH = 6.0, 7.0, and 8.0 the inactivation curves were straight lines as plotted against the time of exposure. At other acidities the inactivation was faster at first, but became slower as the time of exposure increased. The minimum inactivation occurred at pH = 6.0.

This relation to pH was studied further by Morgulis ('30). He disagreed with the opinion of Pincussen that catalase was most injured at its optimum pH = 6.8. Using a Hanovian mercury arc, operating on 110 volts and 5 amperes at a distance of 25 or 50 cm., he was able to show that the greatest injury occurred between pH = 8.0 and 9.0, which is on the alkaline side of the optimum. The stability of his preparation was greatest toward ultra-violet and heat at pH = 6.0, which is on the acid side of the optimum. This pH is suggested as the probable isoelectric point. This paper contains excellent graphs relating activity to pH and time of exposure.

3. Theoretical considerations.—The literature contains a surprisingly great amount of trivial discussion concerning the significance of catalase in tissues. The accumulated authentic data are of permanent value, but their significance is not well understood. A very important statement was published by Pincussen and Tanino ('31), calling attention to two diametrically opposed interpretations of the relation of catalase to respiration. In their study they found a decrease of liver catalase on irradiating the live rats. Assuming that catalase develops proportionally as it is needed to destroy excess hydrogen peroxide, this decrease may be interpreted either as due to an increased oxidation in which hydrogen peroxide is used or to a lessened production of the peroxide by some decreased respirational process.

The paper by Ranjan and Mallik ('31) is one of the most important contributions on the significance of catalase. They show very convincingly that catalase activity parallels the *formation* of hexose, whether this formation results from hydrolysis of disaccharides or from photosynthetic activity.

Wynd ('34) has suggested that this parallelism may signify a transitory peroxide group during the mobilization of the hexose substrate for respiration, and that some of this peroxide is available to peroxidase.

Ostwald ('08), finding that sunlight decreased catalase of larvae when rayed both *in vivo* and *in vitro*, and also incidentally that peroxidase was increased under similar conditions, concluded that these enzymes were connected with phototropism. He also observed that the rayed larvae increased in weight over the controls, but he wisely admitted that these phenomena need not be related necessarily.

The statement of Lockemann, Thies, and Wichern ('08), that the effects of high mountain sun and of sunbaths in general were connected with the depression of the catalase, was made on the unwarranted assumption that *in vivo* effects were similar to those obtained in their *in vitro* raying of blood. The correlation of many apparent discrepancies in the behavior of catalase to irradiation in *in vivo* studies necessitates a consideration of the relative dosages. From the available data it appears that heavy and therefore toxic doses increase catalase of animal tissues while lesser dosages are depressing. The data supporting this generalization are yet comparatively meager and need further verification.

Both of the *in vivo* plant studies show increased catalase. In one instance the rayed seeds produced more vigorous growth, and in the other the growing plants were rayed until they exhibited definite injury. Further data concerning this problem are included in the second paper of this series.

In the *in vitro* studies on animals it is particularly important to separate the catalase activity of hemoglobin and that of the true enzymes, as has been adequately pointed out by Pincussen and Seligsohn ('26). The widely different degrees of injury of catalase solutions by sunlight and by the mercury arc need not be considered incompatible since many known factors, such as dilution, presence of other substances, light intensity, etc., exert powerful influences.

It is clear that catalase is injured by light when irradiated *in vitro*.

B. OXYGENASE

1. Animals.—The general destructive action of ultra-violet on oxygenase of animal origin was reported by Vedder ('24). He found that temperatures greater than 62° C. caused the disappearance of the oxidase-containing granules of leucocytes, but ultra-violet light caused their disappearance in the absence of any temperature change.

The most recent contribution on this subject is that of Wohlgemuth and Sugihara ('25). It had previously been pointed out by Wohlgemuth and Yamasaki ('24) and by Yamasaki ('24) that skin is rich in the enzymes, diastase, lipase, catalase, and oxygenase (phenolase). This oxygenase could oxidize the dihydroxy derivatives of adrenalin, dioxyphenylalanine, and pyro-catechol. Wohlgemuth and Sugihara studied the diastase, lipase, gelatinase, and oxygenase in the skin of different animals and found that oxygenase was highest in the rat and frog. In general, all the animals exhibited greater enzymatic activity of the skin than did man. There was no parallel between the enzymatic content of the blood and that of the skin. Irradiation of guinea pigs over a sufficiently long period with natural sun, artificial sun, and artificial ultra-sun caused a considerable increase of oxygenase, and a decrease of diastase and lipase. In no animal was there any change in the activity of these enzymes in the blood.

2. Plants.—Agulhon ('11) made the observation that the oxygenase (laccase) extracted in glycerin from the fungus *Russula* was extremely stable towards irradiation. Even 6 hours of raying by a Heraeus mercury arc at a distance of 15 to 20 cm. only rarely produced a detectable lessening of its subsequent action on 1 per cent guaiacol. However, if the glycerin extract were diluted with 4 parts of water then oxygenase was notably destroyed in 3 hours of irradiation. Wave lengths longer than 3022 Å were found to be almost inactive.

The following year ('12) he published a more extended report. The method of raying was similar to that of his earlier work, and from the identity of the numerical data it may be

inferred that the previously obtained data were the basis of his later paper. Besides restating the observations of his earlier paper, this publication describes a possible mechanism for the action of the ultra-violet rays. Since oxygenase is not attacked in a glycerin extract in vacuum, and but a slight decrease becomes apparent when the glycerin extract is diluted with water, it is therefore probable that the destructive action is one of oxidation.

It is known that ultra-violet light decomposes water to a slight degree, liberating hydrogen. In the presence of oxygen, this hydrogen forms hydrogen peroxide, which injures the enzyme. Experiments showed that .5 per cent hydrogen peroxide destroys completely the oxygenase of *Russula* extracts in 4 hours. Visible light was thought to owe its destructive action to the same mechanism, although in this instance actual air was necessary, while with the ultra-violet the slight traces of oxygen unavoidably present in vacuum sufficed.

3. Theoretical considerations.—Because of the small amount of work done on the behavior of the oxygenases to ultra-violet, it is difficult to arrive at definite conclusions. If the interpretation of Agulhon is verified, then it forms a significant basis upon which we may interpret various of the activities of ultra-violet light. The authenticity of the formation of hydrogen peroxide by the action of ultra-violet on water is undoubted, but whether this could act destructively on the oxygenases is a question, although the fact that .5 per cent hydrogen peroxide completely inhibited *Russula* oxygenase in 4 hours cannot be ignored. More discussion of the problem will follow in the theoretical consideration of peroxidase.

The work of Wohlgemuth and Sugihara is suggestive, since it is not impossible that the oxygenase increase in the skin of rayed animals might play a role in the development of melanin in spite of the fact that the specific enzyme preparation used in these experiments did not affect tyrosine. The distinction between oxygenase and the tyrosinase may be assailed on theoretical grounds, and the entire problem of the nature of the ac-

tivity of oxygenase and tyrosinase is not as firmly supported as many writers suppose. A review of this interesting problem is not appropriate in the present paper.

C. TYROSINASE

1. Animals.—Narayanamurti and Ramaswami ('29, '30) irradiated the larvae of *Dolichos* and found consistently increased tyrosinase activity of extracts.

It was shown by Lignac ('30) that human skin, after death, would develop melanin as a result of raying with a Kromayer mercury arc. Several hours of exposure of dead skin were needed to produce the same degree of pigmentation as an exposure of 4 minutes of live skin. The difference was attributed to the constant oxygen supply of live skin. One might assume very reasonably that the increase of melanin in skin after raying is due to an increase of the activity of tyrosinase. Pincussen and Hammerich ('31a), acting on this supposition, modified the method of Raper and Wormall for the estimation of tyrosinase, so that it could be used as a micro method. Enzymes prepared from meal worms were rayed *in vitro* with a mercury arc. Tyrosinase, as most other enzymes, was greatly injured, and they concluded that the formation of melanin in the skin by ultra-violet was not due to an increase of tyrosinase.

2. Plants.—The papers of Agulhon ('11, '12), mentioned above, state that the extracts from *Russula* contain tyrosinase and that this enzyme is more stable towards irradiation than is oxygenase. Like oxygenase, it was not destroyed by ultra-violet in glycerin in vacuum, and only slightly injured when rayed in a water solution in vacuum. Likewise, it was inactivated in 4 hours by .5 per cent hydrogen peroxide. It was thought to be destroyed by the same mechanism as suggested for oxygenase.

3. Theoretical considerations.—The conclusions of Pincussen and Hammerich may be attacked on several grounds. In the first place, as has been clearly demonstrated by Fuller ('32), the behavior of an enzyme under *in vitro* raying bears no relation to its behavior when rayed *in vivo*. Moreover, the

total exposure, 1 hour at 50 cm., might well have obscured the effect of less toxic exposures. But the most arresting fact of all is that these authors used dioxyphenylalanine, as well as tyrosine, as a substrate, with identical results. Wohlgemuth and Sugihara found that dioxyphenylalanine was oxidized more by the skin oxidase (called phenolase by them) extracted from guinea pigs which had been rayed while living with a mercury arc. Since enzymes are defined in terms of their substrates, it may be assumed that whatever enzymes actually attack this particular substance, they reacted differently to *in vivo* and *in vitro* irradiation. These authors did not, however, obtain any oxidation of tyrosine by their skin extracts, and yet we know that tyrosinase should have been present in the fraction which they called "phenolase." As mentioned above, the oxygenase and tyrosinase reactions are surprisingly complex and confused in the literature.

It seems reasonable to conclude that tyrosinase, as most enzymes, is injured by irradiation *in vitro,* but that its behavior *in vivo* to exposures has not yet been reported authentically.

D. PEROXIDASE

1. Animals. a. Milk.—The paper of Hertel ('04), already cited as the earliest study on the effect of ultra-violet on catalase, occupies a similar position in respect to peroxidase. Milk peroxidase was studied by mixing 2 cc. of fresh milk with 2 cc. of .1 per cent aqueous solution of guaiacol and 1 drop of hydrogen peroxide. Irradiating the milk with the 2800 Å magnesium line produced a notable diminution of activity even with as brief exposures as 5 minutes.

Römer and Sames ('10) materially extended the observations of Hertel. They used 5 cc. of cow's milk mixed with 2 drops of guaiacum tincture and 6 drops of 2 per cent hydrogen peroxide. If the milk were rayed with a Heraeus mercury arc at a distance of 15 cm. for 1 hour, the peroxidase activity was completely inhibited. Exposures of ½ hour allowed a positive test to appear, although less than the control. When milk whey was rayed, a trace of peroxidase activity remained after 1 hour of exposure, but 1¼ hours were completely inhibitory. These

authors state that this destruction of the peroxidase (called "oxidase" by them) could have no hygienic significance in infant feeding, since human milk normally contains no peroxidase.

In view of the great similarities between cow's milk and human milk, it seems strange that such a difference could exist in this particular. Asakura ('32) has shown that human milk lacks peroxidase if it also lacks vitamin B. Heavy feeding of vitamin B causes the peroxide reaction to appear.

Reinle ('21) extended the study of the peroxidase of cow's milk to include the effect of Röntgen rays, the γ-rays from radium chloride, and ultra-violet. The X-rays and γ-rays had no effect. When the milk was rayed with a mercury arc operating on 110 volts and 4 amperes at a distance of 15 cm., there was a temporary increase for the first 20 to 30 minutes. This stimulation is not significant since mere warming of the milk will also give this effect. This apparent stimulation could not be due to the destruction of the dehydrogenase system by the irradiation, since, as will be shown later, these enzymes are notably stable towards ultra-violet. The effect of warming could very well depend, on the other hand, on the inhibition of dehydrogenating systems. Such an effect has been suggested by Keilin ('29) to account for the fact that fresh baker's yeast exhibits no peroxidase test while warmed yeast does.

1. Animals. b. Tissue.—The relationship between animal tropisms and the light-sensitive enzymes was studied extensively by Ostwald ('08). He found that strong sunlight increased the peroxidase activity of extracts from the larvae of *Porthesia chrysorrhoea.* Irradiation of the living larvae also produced the increase, from which it was concluded that phototropism was connected with cellular respiration. The peroxidase activity of irradiated Paramecia was studied by Roskin and Schischliajewa ('34), but no conclusive results were obtained.

2. Plants. a. In vivo studies.—There have been no data reported on the effect of raying living plants on the activity of

peroxidase. The authors report studies of this problem in the next number of this series.

2. Plants. b. In vitro studies.—The effects of strong sunlight were reported first by Bach ('08). The preparations were exposed in Erlenmeyer flasks, and hence only the longer ultra-violet reached the solution. The exposures lasted from 4 to 76 hours, with a progressive decrease in the activity of the enzyme.

Jamada and Jodlbauer ('08), in an extensive study on the effect of irradiating peroxidase of vegetable origin, reported that horseradish peroxidase was injured by 15-minute exposures to sunlight even when the light was filtered through 20 cm. of water to remove the infra-red. The injurious effect of ultra-violet was shown by the fact that the activity of the enzyme was lessened more by 25-minute exposures to sunlight filtered through quartz than through ordinary glass. Exposures to a mercury arc with 20 cm. of water used as a filter were made. When ordinary glass was interposed there was some injury, but when a quartz plate was substituted the injury was very marked. It was also shown that the visible portion of the spectrum, either from the sun or from a mercury arc, had no effect in the absence of oxygen. The rays from an unscreened mercury arc gave great injury in absence of oxygen, which was a little increased if oxygen were present. From these data the authors concluded that ultra-violet exerted its injurious effect by a fundamentally different mechanism than did the visible light. If eosin were present in concentrations of 1/2000 to 1/5000 N, the peroxidase was sensitized notably towards the visible light. The results after 75 minutes were:

Control..... 0 per cent injury
Rayed.....41 per cent injury
Rayed with eosin....58 per cent injury

This paper contains excellent data illustrating the fact that fluorescent substances may act as sensitizers by changing incident light to more harmful wave lengths, or they may act as protectors by degrading toxic to harmless wave lengths. Thus,

in the present study, eosin and Bengal-red (1/10,000 N) sensitized peroxidase to the visible light, when methylene-blue and the sodium salt of dichloranthracene-disulphuric acid protected the enzyme. In the case of methylene-blue, the injury by visible light was lessened 50 per cent. Eosin in the ultra-violet had no sensitizing action. In the absence of the visible rays, it had a protective influence against ultra-violet, since it absorbed some of the toxic waves and rendered them harmless. In the absence of oxygen the visible could not act, hence eosin also protected.

The fact that Karamitsas ('07) found that eosin did not significantly sensitize peroxidase may be due to his use of thinner glass tubes. If the ultra-violet were transmitted, then eosin would exert a protective action toward these waves which might cancel the added destructive power of the visible, thereby giving the false appearance of inaction on the part of the fluorescent compound.

Bering ('12) reported a stimulation of horseradish peroxidase by irradiation. Exposure to strong sunlight in dosages of .7 to 1.0 "Finsen" units stimulated the subsequent formation of purpurogallin about 6 per cent. The unscreened mercury arc gave only inhibition, but when filtered through a rabbit's skin the rays stimulated peroxidase again about 6 per cent. Experiments with filters showed that the red region of the spectrum had no effect; the yellow and also the green regions were stimulative; the blue plus the long ultra-violet stimulated about 12 per cent. The author concluded that those rays which penetrate the skin stimulate the respiratory enzymes and therefore are physiologically important. Later Bering and Meyer ('12a, b) verified these results, and pointed out that ultra-violet filtered through rabbit's skin stimulated or inhibited peroxidase, depending on the concentration of the enzyme. The unfiltered sunlight stimulated when the exposure was short.

Capelli ('26) rayed a purified vegetable peroxidase and found the mercury arc injurious. The solutions developed ozone which may have caused an initial apparent increase in

oxidative power. During this same year, Meyer ('26) published a review in which he restated the belief that light activated the oxidizing enzymes, and was of therapeutic value on that account. New data were given verifying the stimulation of peroxidase by exposure to the mercury arc, but only when the shorter irradiations were removed by a rabbit's-skin filter.

Pincussen and Hammerich ('31b) prepared a very concentrated horseradish peroxidase according to the method of Willstätter. They mixed .4 cc. of the preparation with .2 cm. of .5 per cent hydrogen peroxide, and then diluted to 400 cc. with water containing 1 gm. of pyrogallol. Aliquots of 20 cc. were removed for the determination of purpurogallin. The reaction was stopped by adding 10 cc. of 10 per cent sulphuric acid. The color was extracted by 20 cc. of ether, and the ether solution compared colorimetrically with a .57 per cent solution of chromic acid. The enzyme solutions were diluted with 5 parts of water. Acetate buffers were used to obtain pH = 3.4, 4.0, 4.6, 5.2, 6.0, and 6.4. The exposures lasted 30 and 60 minutes. The light source was a "Vitaluxlampe" mecury arc with a glass cell to filter out the heat rays. The enzyme was greatly injured by irradiation. The pH had no great influence on the degree of injury, although there was a greater diminution of activity when the solutions were acid.

3. Theoretical considerations.—The effect of raying on peroxidase is subject to several sources of error. The ability of ultra-violet rays to produce hydrogen peroxide in water has been demonstrated by a number of students among whom we may mention Tian ('11). If sub-optimum concentrations of peroxide are used in experiments, this additional substrate for the enzyme may cause an increase in oxidation. This is especially true of oxygenase, in which the slight amount of peroxide formed would enable the otherwise inactive peroxidase to act, obscuring the activity of oxygenase itself. Peroxides may also develop when oils are irradiated, as has been worked out by Delore ('29) and others. These peroxides not only act as an additional source of substrate for the enzyme, but also cooperate with ultra-violet on their own account to ac-

complish oxidation of the dye indicator. The peroxide also is destroyed by ultra-violet, and Koeppe ('30) has shown that the liberated oxygen is in the nascent condition and easily oxidizes guaiacol. The oxygen that develops when hydrogen peroxide is decomposed by heat does not oxidize guaiacol. From Koeppe's data, it is seen that false results may be obtained if the enzyme is irradiated in the presence of the indicator.

The influence of ultra-violet on tautomeric equilibria is well known. It is not improbable that substances which exist in a keto-peroxide tautomeric equilibrium may have oxidative powers due to this fact when affected by irradiation. The existence of an enol and a keto-peroxide form in cholesterin is particularly significant, since Remesow ('32) has shown that this keto form interferes, because of its oxidative power, with the conventional Nadi test for oxygenases.

Pincussen ('30), in the most adequate discussion that has appeared concerning the physiological effects of ultra-violet, has pointed out that the mechanism of color formation in the peroxidase tests is very complex, and that the observed stimulation may not be connected with the enzyme itself. From the discussion above, it is seen that this is indeed probable.

The few instances of stimulated peroxidase activity resulting from irradiation are probably due to some of the disturbing factors discussed above. The theory that light, particularly ultra-violet, owes any of its physiological effect to stimulated oxidizing enzymes is to be disregarded. It is not known whether the thermo-stable peroxidases react differently than the labile type.

E. DEHYDROGENASE

1. Animal tissues.—Krestownikoff ('27) prepared a dehydrogenase solution from horse muscle, using potassium succinate as the hydrogen donater. Evacuated tubes containing the enzyme-succinate-methylene blue mixture were rayed in a water bath by a carbon arc. Since glass tubes were used, only the longer ultra-violet reached the solutions. Exposure to the carbon arc stimulated the rate of reduction of the methylene-

blue. Control solutions containing no enzyme showed no reducing power.

Junghagen ('28) found that the succinic-acid and glycerophosphoric-acid dehydrogenases extracted from horse muscle were rapidly destroyed by ultra-violet irradiation. Bertraccini ('29) found that dehydrogenase of animal origin, as studied by the reduction of m-dinitrobenzol, was increased by small exposures to ultra-violet, but depressed by larger doses.

The work of Palmieri ('33) was more extensive. He rayed rabbit eyes for various periods, and then studied the reducing power of the lenses by Thunberg's method. Immediately after raying, there was a slight increase in the rate of reduction of methylene-blue, followed by a decrease, and finally, 3 days after the irradiation there was a progressive increase.

2. Plants. a. Bacteria.—The only data concerning the behavior of bacterial dehydrogenases depend on inferences drawn from the work on *Bacillus coli,* already quoted, by Cook and Stephenson ('28). These authors studied the relation of the viability of the cell to its ability to oxidize aerobically glucose and its fermentation products. Cell suspensions exposed 20 minutes to a mercury arc contained but 2.74 living cells per 1000, and exposures of 30 minutes killed all but 2 per 1000. The substrates used were glucose, acetate, lactate, and formate. When glucose was the substrate, the cells rayed 20 minutes showed only about one-half as much oxygen use after a period of 30 minutes, but after one hour the total oxygen use was about equal to the control. The same was true of lactate and acetate, and only in the case of formate was an undoubted inhibition of oxygen use evidenced. The authors concluded, as has been mentioned above, that no significant difference was found between the oxidation of dead and living cells. It should be mentioned, however, that the objections to their technique, already described, are pertinent also to this interpretation.

2b. Higher Plants.—The dehydrogenases of plants have not been as well studied as those of animals, and therefore the extensive paper by Lehmann ('22) is of particular importance.

The seeds of *Phaseolus vulgaris* were ground very fine and .05 gm. placed in Thunberg tubes. Besides the meal, each tube received .1 cc. of 1/5000 methylene-blue, .1 cc. of 1/10 N potassium biphosphate as a buffer, .5 cc. of potassium malate as a hydrogen donator. Water was added to give a total of 1 cc. The potassium malate solution was made by dissolving 346 mgms. in 10 cc. of water. The tubes were evacuated and the time required for the reduction of the methylene-blue recorded. The dry powder was rayed with a carbon arc at a distance of 40 cm., with the following results:

Control .....required 21 minutes for reduction
Rayed 100 minutes....required 19 minutes for reduction
Rayed 240 minutes....required 43 minutes for reduction

It is significant, as will be pointed out later, that a stimulation occurred when the sample was rayed 100 minutes.

3. Xanthine dehydrogenase.—Some authors consider the aldehyde oxidizing system in milk to be catalyzed by an enzyme distinct from that concerned in the oxidation of xanthine. It seems logical that substrates differing from each other as greatly as do aldehyde and xanthine should be activated by different enzymes. Most writers believe, however, that a single enzymic system is concerned.

Römer and Sames ('10), in the paper referred to above, reported the effect of raying cow's milk on the dehydrogenase (reductase). They used methylene-blue as an indicator in the presence of formaldehyde. Exposures to a Heraeus mercury arc at a distance of 15 cm. for various periods gave a small increase at shorter exposures and then a decrease, although even 2 hours of irradiation did not completely destroy the enzyme, as is shown by the table:

Control .....reduced methylene-blue in 6 minutes
Rayed ½ hour.....reduced methylene-blue in 4 minutes
Rayed 1 hour.....reduced methylene-blue in 6 minutes
Rayed 2 hours.....reduced methylene-blue in 30 minutes

This same reaction was studied by Reinle ('21) and the resistance of the dehydrogenase to ultra-violet verified. His abbreviated results are:

Fresh milk.....	reduced methylene-blue in	7½ minutes
Rayed 10 minutes.	reduced methylene-blue in	8 minutes
Rayed 30 minutes.	reduced methylene-blue in	12 minutes
Rayed 1 hour.....	reduced methylene-blue in	15 minutes
Rayed 2 hours....	reduced methylene-blue in	19½ minutes
Rayed 3 hours....	reduced methylene-blue in	24 minutes
Rayed 4 hours....	reduced methylene-blue in	31 minutes

Injurious effects of the same order of magnitude were again obtained by Pincussen and Oya ('29). They mixed 1 part fresh cow's milk with 1 part of M/3 phosphate buffer and 1 part of water, and added 5 cc. of this mixture to .3 cc. of a methylene-blue solution. The methylene-blue solution was prepared by mixing 5 cc. of saturated alcoholic methylene-blue with 5 cc. of formalin, and diluting to 200 cc. with water. The milk-buffer mixture was rayed in thin layers at a distance of 20 cm. from a mercury arc for 1 hour. The dehydrase (aldehydrase) was weakened about 50 per cent.

Bernheim and Dixon ('28) studied the effect of light on xanthine oxidase of fresh milk, the caseinogen preparation of Dixon and Thurlow, and on the whey preparation of Dixon and Kodama. Hypoxanthine or aldehyde were the hydrogen donators, and methylene-blue or nitrate were the hydrogen acceptors. The reduction of the methylene-blue was followed in Thunberg tubes. Moderate irradiation by a carbon arc or by a 100-watt filament bulb gave great stimulation when traces of oxygen were present. Long exposures were inhibitory. Both of these effects were found to be due to the formation of traces of hydrogen peroxide. The first stage of oxidation of the oxidase by the peroxide caused an increase in its activity, while its further oxidation lessened its activity. This interpretation is made probable by the fact that 1/100,000,000 M hydrogen peroxide gave stimulation. Higher concentrations than these destroy the enzyme. The intensity of the effect depends on the amount of enzyme present.

4. Theoretical considerations.—It is interesting to note that small increases of dehydrogenating activity, when the period of irradiation was short, were observed in milk by Römer and Sames and by Krestownikoff, and in animal tissues by Bertraccini, by Krestownikoff, and by Palmieri.

Despite the few published papers on the subject, it is evident that dehydrogenases are particularly resistant to ultra-violet destruction. It would be interesting to compare the resistance of the oxidases under similar *in vivo* conditions. The work on xanthine dehydrogenase (known more commonly as "xanthine oxidase") furnishes the most convincing evidence at hand of the stimulation of an oxidizing enzyme by ultra-violet light. The fact that methylene-blue can act as the hydrogen acceptor in the absence of oxygen, and that formaldehyde can be substituted for xanthine or hypoxanthine as the hydrogen donator suggests an explanation for the several instances of dehydrogenase stimulation described above. In many of these experiments, the preparations, particularly those of indefinite composition, may have been exhibiting the typical xanthine-oxidase reaction rather than that of the supposed dehydrogenases. This seems the more probable when it is recalled that xanthine and its derivatives, and xanthine- activating systems, are widely distributed in nature.

Meldrum ('33) has summarized the characteristics of this interesting enzyme. He points out that it differs from the usual dehydrogenases in that the dehydrogenase inhibitors, such as vanillin, urethane, diethylurea, propionirile, etc., do not lessen its activity, nor is it inhibited by hydrogen cyanide or hydrogen sulphide. It contains neither cytochrome nor an oxidase. From these facts it is seen that the enzyme is fundamentally different from dehydrogenase of the succinate dehydrogenase type.

V. Sulphydryl Compounds

A. Experimental

The first reversibly oxidizable substance of known composition to be isolated from tissues was glutathione. In later years

it has assumed great importance in respiration chemistry, although comparatively few students have studied it in connection with the problems of ultra-violet biology.

Glutathione bears an intimate relationship to many enzyme systems, and it has also been considered to be a factor concerned in the proliferation of cancer tissue. In view of the effect of raying cancer tissue with ultra-violet, Woodward ('33) irradiated glutathione in pure solution. She used a Uviarc at a distance of 25 cm., and irradiated the solution in thin layers on an ice bath to eliminate destruction by the heat. The destruction of the glutathione, as well as the oxidation of sulphydryl group generally, was found to depend on the pH, on the time of exposure, and on the concentration of the sulphydryl compound. At pH = 7.0, the oxidation of the sulphydryl compound to the disulphide proceeded more rapidly than destruction, and this process is therefore probably a primary step in the destructive effect of ultra-violet on glutathione. The destruction seemed to be a progressive oxidizing effect rather than the liberation of inorganic sulphide or free sulphur.

Bersin ('33) based his study of the sulphydryl compounds on the fact that papain had been shown to be activated by hydrogen sulphide or by such thiol compounds as cystein or glutathione. This activation involves a reduction. In opposition to Woodward, Szendro had shown that cystein developed from cystine when exposed to ultra-violet light. The work of Szendro was repeated by Bersin ('33) and found to be correct. He rayed the substance, $HOOC.CH(CH_3).S.S.C(CH_3).COOH$ and found that it went to the reduced form. This experiment took place in a hydrogen atmosphere, while Woodward irradiated her solution in air. Bersin assumed that papain was a disulphide, PaSSPa, which became activated when reduced to the PaSH form by irradiation in hydrogen.

Pincussen and Takahashi ('33) removed the organs from animals 30 minutes after raying them and determined their gluthathione content. These authors irradiated with infrared, blue, orange-red, and yellow. The latter was obtained by a sodium-vapor lamp and a significant amount of ultra-violet

was present. The sodium lamp produced in general a small increase in the glutathione content, with no significant difference when the ultra-violet was removed by filtration.

Wels and Jokisch ('33), Wels ('33), and Wels and Hesse ('34) have shown that egg albumen when rayed in nitrogen with ultra-violet developed a reversibly oxidizable body, probably a sulphydryl compound. It is very probable that irradiation of living tissue produces some similar result which cannot but be of importance to the respiratory processes.

B. THEORETICAL CONSIDERATIONS

The apparently contradictory results of Woodward and Bersin are to be explained by the fact that Woodward rayed her solution in air, while Bersin used a hydrogen atmosphere. Wels and his co-workers state that when egg albumen is rayed in air, the oxidized form of the reversible oxidizable body occurs, while in nitrogen it is the reduced form.

This difference of effect, depending on the presence of oxygen, has a possible explanation in the fact that ultra-violet rays cause water to split off hydrogen in small quantities. If air be present, then hydrogen peroxide results, and this peroxide is responsible for an oxidizing effect. In the absence of oxygen, this "active" hydrogen could be taken up by any reducible body, causing reduced forms to develop. While these reactions undoubtedly occur, there is also the direct effect of the ultra-violet itself to be considered. Hence, the ultra-violet may cause the reversibly oxidizable body to develop from protein, but the presence or absence of oxygen would determine, by the above suggested mechanism, whether this substance would appear in the oxidized or reduced state.

VI. Conclusions

A review of the published data concerning the effect of ultra-violet on oxidizing enzymes *in vitro* shows, with the exception of xanthine oxidase, generally destructive effect. Agulhon ('12) has described three classes of enzymes depending on the part that ozygen plays in their photodestruction.

1. Invertase, oxygenase (laccase), and tyrosinase are destroyed only in the presence of oxygen by visible rays and are less rapidly attacked by ultra-violet in vacuum, the rapidity depending on the amount of hydrogen peroxide that develops.

2. Catalase and emulsin are destroyed in vacuum by all radiations, but less actively in vacuum than in oxygen.

3. Rennet is insensible to the visible radiations, but is attacked by ultra-violet with equal intensity in vacuum and in oxygen.

Are these three classes of enzymes dependent on their inherent differences, or are these divisions only apparent, depending on some activity of impurities, or on the nature of the substrate? To these questions Agulhon wisely replies that the present state of knowledge is too incomplete to allow definite answers.

The various degrees of injury reported by different workers are not to be considered as inconsistent nor surprising, since many factors both known and unknown are cooperating to give the finally observed effect. This is particularly true of studies carried out *in vivo*.

Despite the efforts of a few writers to correlate physiological phenomena due to raying with the effects on individual enzymes, it may be said that such correlations are based upon very insecure evidence. The profound difference between raying enzymes *in vivo* and *in vitro* itself argues against any such conclusion. The few data reporting similar reactions *in vivo* and *in vitro* of peroxidase and catalase require further verification.

Finally, we must conclude that it is not possible at the present time to analyze the effect of ultra-violet light on respirational metabolism into its various components.

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STUDIES IN ULTRA-VIOLET AND RESPIRATORY PHENOMENA. II

The Effects of Ultra-Violet on Respiration and Respiratory Enzymes of Higher Plants[1]

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The present paper reports observations on the respiratory rate and activity of oxidizing enzymes of tomato and bean plants as affected by ultra-violet radiation, and offers suggestions concerning the relationship of oxygenase and peroxidase to the respiratory mechanism. The pertinent literature has been discussed in the first paper of the series (Wynd and Reynolds, '35).

EXPERIMENTAL METHODS

From several hundred plants of Bonny Best tomatoes and Red Kidney field beans, those of uniform size and vigorous physiological condition were selected. They were irradiated under the conditions of the individual experiments by a Burdick quartz mercury vapor arc suspended above the plants. The distances reported were measured from the arc to the surface of the soil. Two types of filters were used: one a quartz-plate

[1] The investigations reported in this paper were carried on through aid from the Science Research Fund of Washington University, St. Louis, provided by the Rockefeller Foundation.

tray 5 mm. thick, containing 2 cm. of distilled water; the other, a tray made of Vita glass, containing a similar depth of water. The quartz water cell, according to Jones ('28), absorbs all wave lengths longer than 1.4 μ, and the Vita glass water cell eliminates not only this infra-red but also the ultra-violet shorter than 2894 Å.

Respiration was determined by weighing the amount of carbon dioxide eliminated per gram of fresh weight over a given period (Wynd, '32, '35). The intrinsic accuracy of this apparatus is very great. However, the respirational rates of the plants themselves were found to vary about ± 5 per cent in duplicate controls, and so this must be regarded as the maximum accuracy of the data obtained. The plants were cut off at the surface of the soil in the evening and placed in beakers of water in the respiration chambers, and the next morning the carbon dioxide absorption bulbs were weighed. Five plants were used for each determination, and observations on the control and the experimental plants were carried on simultaneously.

Since all of the enzymatic determinations were made on juice pressed from the plants *after* they had been rayed, the values obtained represent *in vivo* reactions. The necessary distinction between *in vivo* and *in vitro* experiments has been particularly pointed out by Fuller ('32) and by Wynd and Reynolds ('35). The upper leaves and the upper 2 inches of the stems were ground to a paste in a mortar with a little quartz sand, then squeezed through 2 layers of cheese-cloth, and aliquots of this solution used for the enzymatic determinations. Since the usual methods of measuring acidity are disturbed by oxidizing enzymes or by hydrogen peroxide, the final pH of all experimental solutions was checked by the glass electrode.

Extended experimentation by Wynd has shown that the determination of catalase activity can be more closely duplicated by titration than by gasometric methods. However, the gasometric method is entirely satisfactory as a *micro* procedure if the usual precautions are taken. Experiments involving relatively large amounts of material can be duplicated by titration with an error of 1 or 2 per cent, which is not possible by *macro* gasometric procedures. The experimental solutions contained

50 cc. of phosphate buffer at pH = 7.00, 20 cc. of 3 per cent commercial hydrogen peroxide, and 1 cc. of the expressed juice. The reaction time varied from 8 to 20 minutes in the different experiments, and it was stopped by adding 20 cc. of a 25 per cent solution of sulphuric acid. The time of adding the plant juice and of the subsequent addition of acid was carefully controlled by a stop watch. The amount of hydrogen peroxide remaining was then titrated with N/5 potassium permanganate. The reaction times of all the solutions were spaced 1 minute apart to allow time for the pipettings.

Peroxidase was determined by the method of Guthrie ('31). The substrate for the enzyme was prepared by adding 200 cc. of a citrate buffer at pH = 4.5 to 200 cc. of water. To this were added 1 gm. of p-phenylenediamine hydrochloride and 20 cc. of a 4 per cent solution of alpha-naphthol in 50 per cent alcohol. The final mixture was filtered and used immediately. At pH = 4.5, atmospheric oxidation of the substrate is slow, and catalase is so far depressed as not to seriously interfere. The experimental solutions consisted of 25 cc. of substrate, 1 cc. of commercial 3 per cent hydrogen peroxide, and .5 cc. of the plant juice. After 10 to 20 minutes, depending on the intensity of the color which developed, the reaction was stopped by the addition of 5 cc. of a .1 per cent potassium cyanide solution. Since the cyanide inhibits the enzyme but does not interfere with the atmospheric oxidation, the indophenol produced was determined as quickly as possible. This was done by shaking each experimental mixture with 50 cc. of toluene until the water layer was clear. The upper layer of toluene, after being poured off and centrifuged to remove bubbles and tissue fragments, was examined in a colorimeter. The error of this method is about 3 per cent.

Oxygenase was determined similarly to peroxidase except that the hydrogen peroxide was not added to the substrate. Due to the feeble activity of oxygenase as compared with that of peroxidase, 1 cc. of plant juice was used for each determination, and the reaction time was usually extended to 20 minutes. The indophenol was extracted in 25 cc. of toluol. The error of this method is about 3 per cent.

Since the temperature, reaction periods, etc. varied from day to day, the absolute values of data obtained at different times may not be directly compared, and the tables contain values calculated as percentages of their corresponding controls. These percentage values obtained at different times may be compared directly. In the figures, the numbers on the ordinate represent the per cent of stimulation or depression, the graphical value of the control therefore being zero. The horizontal line at zero divides the stimulation from the depression values. The numbers on the abscissa represent minutes of daily exposure.

EXPERIMENTAL RESULTS

Experiment I.—Bonny Best tomato seeds were planted in greenhouse flats January 23, 1934, and were transplanted to 4-inch pots February 8. The raying began March 4, and was continued daily for 5 days. The distance was 24 inches and the dosages 4, 8, 16, and 32 minutes daily. The unscreened mercury arc was used as a source of illumination.

TABLE I

ENZYMATIC ACTIVITY OF TOMATO PLANTS RAYED AT 24 INCHES WITH THE UNSCREENED MERCURY ARC, CALCULATED IN PERCENTAGES OF THE CONTROL

	Control	Daily Dosage			
		4 minutes	8 minutes	16 minutes	32 minutes
After 5 days of irradiation					
Oxygenase	100	96	107	96	78
Peroxidase	100	97	159	166	213
Catalase	100	123	134	119	125
5 days after irradiation ceased					
Oxygenase	100	95	92	88	74
Peroxidase	100	189	263	345	385
Catalase	100	115	146	194	165

All plants showed definite injury at the end of the 5-day period of irradiation. The plants submitted to the heavier

dosages were very severely burned. One set of determinations was made at the end of the irradiation period and another 5 days later. The plants were not rayed during this intervening period. From table I and figs. 1 and 2, it is seen that with increasing amounts of daily irradiation the peroxidase activity greatly increased. Catalase also increased but less than the

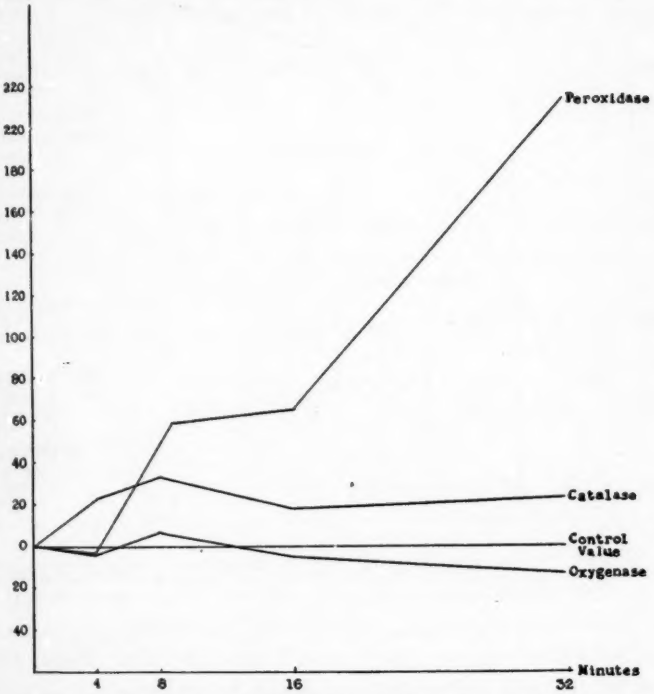

Fig. 1. Determination of enzymatic activities of tomato plants after being irradiated daily for 5 days at 24 inches, through quartz water-cell filter.

peroxidase, while oxygenase was progressively inhibited. These results were of greater magnitude 5 days after irradiation ceased.

Experiment II.—Bonny Best tomatoes were planted February 15, 1934, and transplanted from the flats to 4-inch pots on

April 5. The raying began April 16, at a distance of 100 inches, and continued in 10-minute doses daily for 5 weeks. According to Fuller ('31), these are the optimum conditions for growth stimulation. Some of the plants were rayed through the quartz

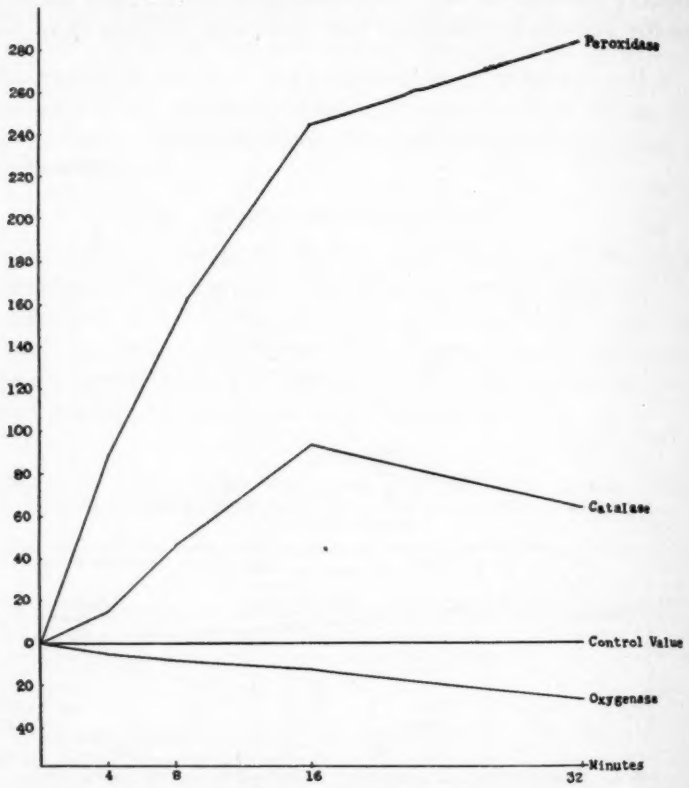

Fig. 2. Determination of enzymatic activities of tomato plants 5 days after irradiation ceased.

water cell, and others through the Vita glass water cell. Two determinations of enzymes and respiration were made on consecutive days at the end of the period of irradiation.

The data in table II, graphically represented in fig. 3, indicate that the plants rayed through the Vita glass water cell

TABLE II

RESULTS OF IRRADIATING 5 TOMATO PLANTS AT 100 INCHES FOR 10 MINUTES DAILY FOR 5 WEEKS, CALCULATED IN PERCENTAGES OF THE CONTROL

Treatment	Fresh wt. in gms. of tops		CO_2 elimination		Relative percentage of enzymatic activity		
	Actual	Relative	Per gm. fresh wt.	Relative	Catalase	Oxygenase	Peroxidase
One day after irradiation ceased							
Control	95.6	100	.00098	100	100	100	100
Rayed through Vita glass water cell	107.5	112	.00128	131	164	104	100
Rayed through quartz water cell	93.5	98	.00102	104	190	117	103
Two days after irradiation ceased							
Control	84.5	100	.00200	100	100	100	100
Rayed through Vita glass water cell	102.0	121	.00218	109	142	103	100
Rayed through quartz water cell	90.0	107.0	.00208	104	118	106	102

were stimulated in growth from 12 to 21 per cent. Since only 5 plants were used for the determination, this result is not statistically significant, but there is qualitative agreement with the results published by Fuller ('31) on the stimulation of growth under these conditions. Respiration is also increased. Catalase showed a very great increase, while oxygenase was but slightly increased, and peroxidase was not affected. The variations given for oxygenase probably are not far beyond the experimental error. There was qualitative agreement between the data obtained on successive days, and consequently on different samples of tissue.

Irradiation through the quartz water cell, which allows much of the short, injurious ultra-violet rays to pass, had no con-

sistent effect on growth, although, as noted above, the number of plants was too small to present convincing evidence. Respiration was stimulated only slightly, less than when the Vita glass water cell was used as the filter. Again catalase was greatly stimulated, while oxygenase showed a smaller increase. Peroxidase was slightly increased, although the increase is too close to expect experimental error to be accepted as significant. It is thus seen that the physiological effects of rayings through

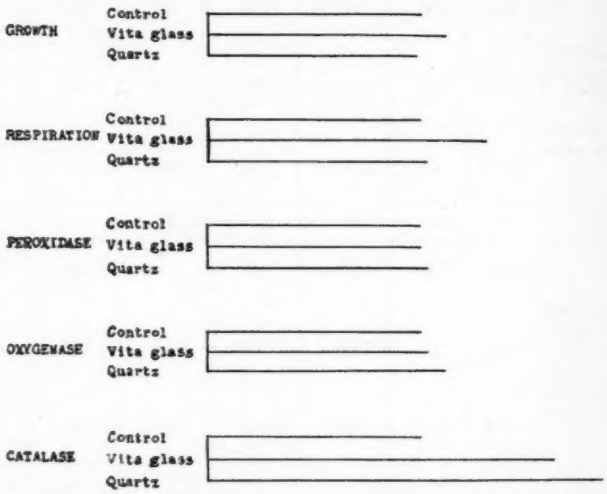

Fig. 3. Determinations made on tomato plants day after irradiation ceased. Plants had been irradiated at 100 inches for 10 minutes daily for 5 weeks. Linear values calculated as percentages of the control.

the quartz water cell were very similar to those obtained by the use of the Vita glass water cell, with an indication of a possible injury of the plants.

Experiment III.—The tomato plants used for this experiment were planted February 15, 1934, and transplanted from the flats to 4-inch pots on April 5. The raying began April 20, at a distance of 36 inches from the arc. The quartz water cell was used as a filter. One group was rayed 15 minutes, and another group 30 minutes, daily for 7 days. Determinations

were made daily for 4 days following the irradiation period. The data are included in table III, and are represented graphically in figs. 4–7 inclusive.

All of the rayed plants in this experiment were greatly injured. The first set of determinations, made the day after the

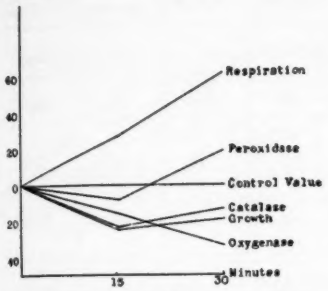

Fig. 4. Determinations made on tomato plants after being rayed at 36 inches for 7 days with quartz water-cell filter.

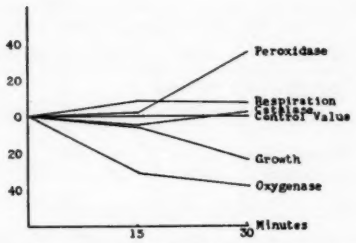

Fig. 5. Determinations made on tomato plants on the second day after irradiation ceased.

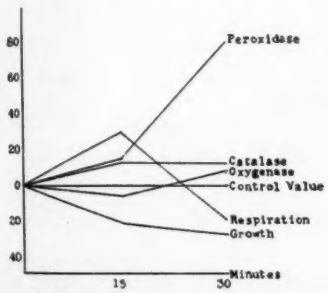

Fig. 6. Determinations made on tomato plants on the third day after irradiation ceased.

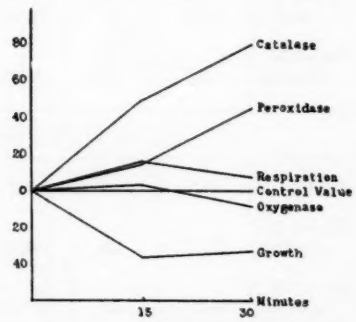

Fig. 7. Determinations made on tomato plants on the fourth day after irradiation ceased.

irradiations ceased, showed a heightened rate of respiration that was proportional to the length of exposure. On subsequent days the amount of carbon dioxide eliminated per gram of fresh weight remained at the heightened level in those plants rayed for 15 minutes daily, but there was a great drop in respiration on subsequent days in those plants which

TABLE III

RESULTS OF IRRADIATING 5 TOMATO PLANTS THROUGH QUARTZ WATER CELL AT 36 INCHES DAILY FOR 7 DAYS, CALCULATED IN PERCENTAGES OF THE CONTROL

Treatment	Fresh wt. in gms. of tops		CO_2 elimination		Relative percentage of enzymatic activity		
	Actual	Relative	Per gm. fresh wt.	Relative	Catalase	Oxygenase	Peroxidase
First determination							
Control	60.2	100	.00130	100	100	100	100
Rayed 15 minutes	46.5	77	.00165	127	77	85	92
Rayed 30 minutes	49.0	81	.00209	161	87	67	119
Second determination							
Control	57.4	100	.00219	100	100	100	100
Rayed 15 minutes	53.9	94	.00235	108	75	67	102
Rayed 30 minutes	43.8	76	.00223	107	104	62	135
Third determination							
Control	80.4	100	.00174	100	100	100	100
Rayed 15 minutes	64.2	79	.00208	129	117	94	115
Rayed 30 minutes	58.7	73	.00153	89	112	108	179
Fourth determination							
Control	86.7	100	.00228	100	100	100	100
Rayed 15 minutes	54.4	63	.00264	116	149	103	115
Rayed 30 minutes	59.1	67	.00248	109	180	91	145

had been rayed for 30 minutes daily. This drop is only apparent, because there were progressively larger amounts of withered and dead tissue in these greatly injured plants on each succeeding day. Since these non-respiring tissues were unavoidably included in the recorded fresh weight, a false value is obtained. This was not an important source of error in those

TABLE IV

RESULTS OF IRRADIATING 5 BEAN PLANTS AT 70 INCHES FOR 10 MINUTES DAILY FOR 5 WEEKS, CALCULATED IN PERCENTAGES OF THE CONTROL

Treatment	Fresh wt. in gms. of tops		CO_2 elimination		Relative percentage of enzymatic activity	
	Actual	Relative	Per gm. fresh wt.	Relative	Peroxidase	Catalase
First determination						
Control	92.5	100	.0053	100	100	100
Rayed through Vita glass water cell	74.5	81	.0053	100	150	91
Rayed through quartz water cell	59.8	64	.0049	92	237	100
Second determination						
Control	70.0	100	.0062	100	100	100
Rayed through Vita glass water cell	58.0	83	.0057	92	119	95
Rayed through quartz water cell	45.0	63	.0068	109	145	108

plants rayed 15 minutes daily. Since the enzymes were determined in the extracted sap, these values represent more truly the comparative activities of the actually living tissue. The peroxidase, with the exception of a single determination, followed the upward trend of respiration. Oxygenase was greatly decreased during the first 2 days after the irradiations ceased,

the decrease being proportional to the length of exposure, but normal values were regained on the third and fourth day. Catalase showed a progressive increase for the four days following the termination of the raying. The activity of catalase shows no apparent relationship to respiration nor to the activities of peroxidase and oxygenase.

Experiment IV.—For this experiment, Red Kidney field beans were planted in flats January 23, 1934, and transplanted

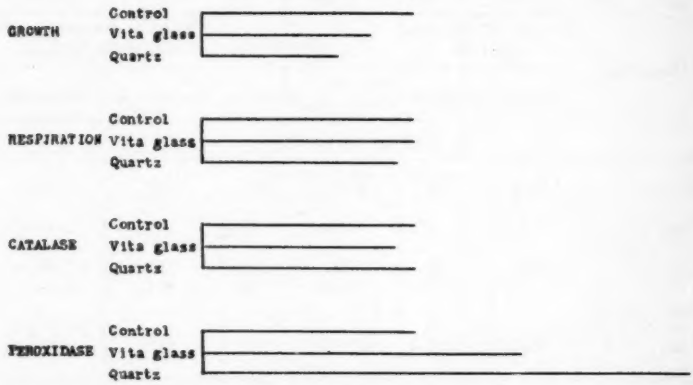

Fig. 8. Determinations made the day after irradiation ceased on bean plants rayed at 70 inches for 10 minutes daily for 5 weeks. Linear values calculated in percentages of the controls.

into 4-inch pots February 8. The irradiation began February 15, and continued for 5 weeks. The distance was 70 inches, and the daily exposure was 10 minutes. One group was rayed through the quartz water cell, and one through the Vita glass water cell. One set of determinations was made the day after the irradiations ceased, and another 4 days later. The results are presented numerically in table IV and graphically in figs. 8 and 9.

All irradiated plants showed injury. The injury and stunting were slight in those plants rayed through the Vita glass water cell, but quite pronounced in those rayed through the quartz water cell. Respiration and catalase were not signifi-

cantly affected, but peroxidase was very markedly increased in both rayed groups, particularly when the quartz water cell was used as the filter. Five days after irradiation ceased these differences were still evident although of lesser magnitude. The tomato plants in experiment II were rayed under approximately similar conditions, but they were stimulated in growth, respiration was slightly increased, peroxidase was insignificantly affected, and catalase was very greatly stimulated.

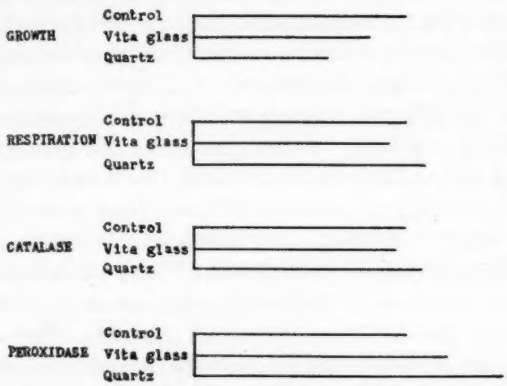

Fig. 9. Determinations made 5 days after irradiation ceased on bean plants rayed at 70 inches for 10 minutes daily for 5 weeks. Linear distances calculated in percentages of the controls.

DISCUSSION

These experiments on irradiated tomato plants show that the rate of respiration as indicated by the amount of carbon dioxide eliminated per gram of fresh weight markedly increases proportionally to the extent of injury. However, it is not possible to obtain accurate numerical data on plants in the most advanced state of injury because the development of dead, non-respiring tissue confuses the weights. This increased rate of respiration is approximately paralleled by the activitiy of peroxidase in the expressed juice. Oxygenase, on the other hand, is inhibited proportionally to the extent of raying. Experiment III shows that normal oxygenase activity is regained

soon after irradiation stops, although the plants remain in a very disturbed physiological condition. Catalase reacts variously. In experiment I it was stimulated, yet in experiment III determinations made the day after raying ceased gave subnormal values. On successive days its activity increased until a considerable degree of stimulation was reached. These results agree with those of Fuller ('32).

The irradiated bean plants reacted somewhat differently. Their growth was not stimulated under conditions stimulative to tomatoes. In experiment IV, the only decided effect was a greatly stimulated peroxidase activity, while the tomato plants under the approximately similar conditions of experiment II showed a greatly stimulated catalase activity as the chief physiological reaction. Bean plants do not give the colorimetric test for oxygenase, and this is itself sufficient evidence that the physiology of beans is different from that of tomatoes. It is not surprising, then, that beans and tomatoes react differently in regard to other features of their physiology.

The data obtained in these experiments have a bearing on the nature of the oxidation processes in plants. The numerous contributions of Bach and Chodat on the intracellular oxidation mechanism have been responsible for the concept that oxygenase unites molecular oxygen to some cellular component to form a peroxide. This peroxide is then activated towards some oxidizable substance in the protoplasm by peroxidase. It is obvious that if this relationship be true, then oxygenase and peroxidase would necessarily be related quantitatively to each other. This is indeed suggested by numerous experiments upon diseased plant tissues in which both enzymes were markedly greater in activity than in normal plants. A summary of these experiments has been published by Wynd ('34).

The necessity of oxygenase for the respiratory process is, however, open to several serious objections. Onslow ('21, '31) has shown that only about 60 per cent of the higher green plants give the oxygenase reaction and that any fundamental component of the respiratory mechanism would be more universally distributed. Kertesz ('34) has shown that fresh tis-

sue of some varieties of peaches does not discolor upon exposure to the air. Qualitative and quantitative studies showed the lack of catechol compounds in the non-discoloring varieties. This indicates that the oxygenase-catechol system may be absent even in very closely related plants, and consequently could not be of fundamental importance.

Wynd ('34) has shown, in an extended study of the tomato plant, that there is no quantitative relationship between the activities of oxygenase and peroxidase. Plants stimulated to great respirational activity by the addition of potassium iodide to the nutrient solution showed a corresponding increase of peroxidase but oxygenase was greatly inhibited. These results, together with those of the present paper, tend to indicate that the oxygenase-catechol-peroxidase system does not represent a fundamental respiratory mechanism in the tomato plant.

SUMMARY

1. Tomato and bean plants were rayed under various conditions by a mercury arc, and the effect on respiration and oxidizing enzymes determined.

2. In tomato plants, *injurious* raying stimulated respiration and peroxidase. Oxygenase was inhibited, and catalase reacted variously but in general was stimulated. The most pronounced effect of *non-injurious* raying was greatly stimulated catalase.

3. In bean plants, comparatively weak dosages produced greatly stimulated peroxidase as the chief reaction. Bean plants did not exhibit oxygenase activity, which indicates a different chemical physiology consistent with their different reactions to ultra-violet light.

4. The comparative activities of peroxidase and oxygenase show that the oxygenase-peroxide-peroxidase system as proposed by Bach and Chodat does not represent a fundamental respiratory mechanism in the tomato plant.

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STUDIES IN ULTRA-VIOLET AND RESPIRATORY PHENOMENA. III

The Influence of Various Regions of the Spectrum on the Anaerobic Fermentation of Yeast[1]

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As was pointed out in the first paper of this series,[2] the claim that ultra-violet irradiation stimulates the fermentation of yeast is based on very insecure foundations. The recent revival of this claim warrants a new and more critical approach to the problem. The present paper reports the results of an attempt to duplicate the essential features of the work of Owen[3] but under more accurately controlled experimental conditions.

EXPERIMENTAL PROCEDURE

The CO_2 elimination was determined by observing the positive pressure developing in the Barcroft differential manometer. Similar vessels were attached to each arm, one vessel receiving the yeast suspension while the other remained empty. Both were thoroughly swept out with nitrogen and the manometer taps immediately closed. After shaking for 30 minutes in

[1] The investigations reported in this paper were carried on through aid from the Science Research Fund of Washington University, St. Louis, provided by the Rockefeller Foundation.

[2] Wynd, F. Lyle, and Reynolds, E. S. Studies in ultra-violet and respiratory phenomena. I. Review of work published before June, 1935. Ann. Mo. Bot. Gard. **22**: 771–835. 1935.

[3] Owen, W. L. Ultra-violet irradiation stimulates yeast activity. Food Industries **5**: 252–254. 1933.

the water bath, the taps were opened and the manometer liquid allowed to come to equal levels in each arm. The taps were then closed, and the positive pressure plotted by frequent readings.

The vessels were of the type shown in fig. 1. The total volume was about 12 cc. Chamber A received the culture suspension and Chamber B an alkali when CO_2 absorption was desired. A metal shield protected the manometer from the heat of the mercury arc.

The water bath was of the Warburg type in which the temperature was held to 25° C., ± .001° C. by a sensitive Thyratron

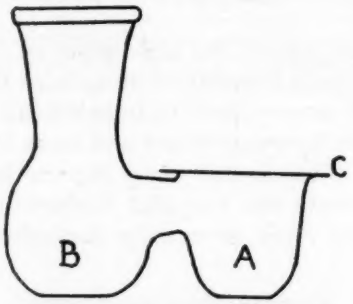

Fig. 1. Respirometer vessel: A, culture chamber; B, alkali chamber; C, quartz cover glass.

relay. The manometers were shaken at the rate of 100 excursions of 4 cm. in length per minute.

The source of irradiation was a Burdick quartz mercury arc suspended immediately above the respiration vessel and removed 25 cm. from the thin quartz plate C. The quartz plate C was immersed 3 cm. below the surface of the water of the water bath. A conveniently built rack allowed the various filters to be placed several cm. above the water surface. A quartz tray, containing water 1 cm. deep, was suspended just below the arc itself. This was necessary to prevent the heat from the arc from cracking the filters, some of which were very fragile.

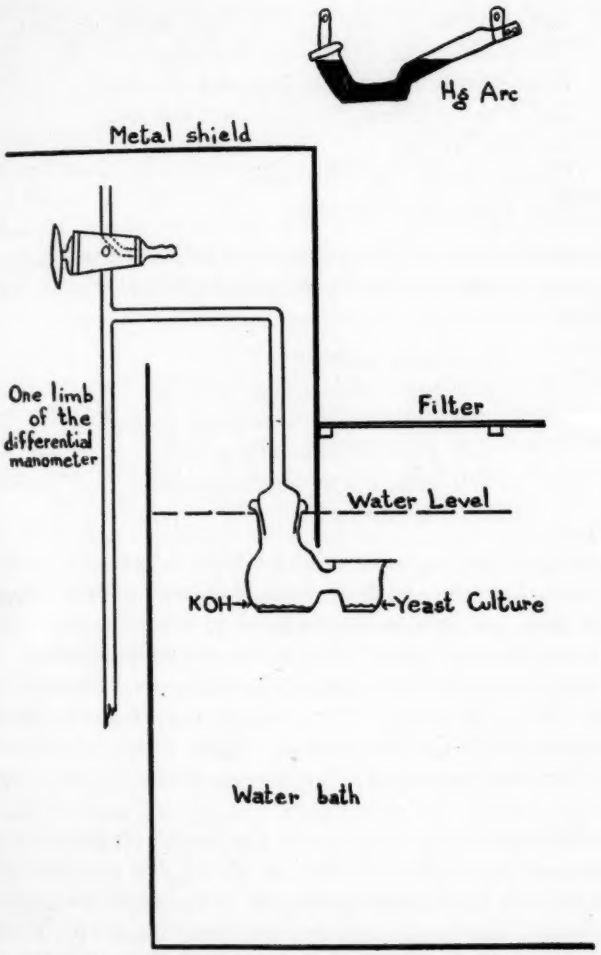

Fig. 2. Arrangement of the apparatus for raying yeast cultures. The alkali is omitted in fermentation experiments.

Through this set-up also the infra-red radiations were eliminated. The following light filters were used:

Red Purple Corex A, No. 986—about 4.0 mm.
Chemical Pyrex Plate—2.1–2.6 mm.
Blue Purple Ultra, No. 585—4.4–4.6 mm.
Red Purple Ultra, No. 597—4.5–5.4 mm.
Nultra, No. 385
Noviol Shade "O," No. 306
Vita glass—2.6–3.0 mm.

The transmissions of these filters are indicated in fig. 3.

Pasteur's yeast medium was used as the substrate. Its composition was:

150.0 gms. glucose
10.0 gms. ammonium tartrate
2.0 gms. mono-potassium phosphate
0.2 gms. calcium phosphate
0.2 gms. magnesium sulphate
1000.0 cc. water

Since autoclaving causes this medium to turn brown if sugar is present, aliquots of 100 cc. were sterilized without the sugar, and 15 gms. of glucose were added just before use. Fleischman's commercial baker's yeast was obtained in pound cakes, only the center portion being used, and 7 gms. of it were seeded in the 100-cc. portions. The seeded medium was vigorously aerated at 25° C. for 30 minutes. Three drops, or about 0.1 of a cc., were then delivered into chamber A of the vessel shown in fig. 1.

The cultures were not pure in the bacteriological sense, but preliminary tests showed that at 25° C. the medium allowed no significant bacterial growth during the short time that each experiment lasted. As may be seen from the graph in figure 4 for the control culture, the fermentation curve over the period represented was practically a straight line. This period is the second hour of the life of the culture, since half an hour was spent in the preliminary aeration, and half an hour in temperature equilibration.

Each experiment was repeated three times with identical results. Almost every determination was made on a separate cake of yeast, thus eliminating the possibility of the results being due to some specific physiological condition of the sample.

RESULTS

No effect on anaerobic fermentation was produced by the irradiation passed through any of the following filters: Blue Purple Ultra No. 585; Red Purple Ultra No. 597; Nultra No. 385; Noviol Shade "O," No. 306. All negative experiments were duplicated with a total of only 1.5 cm. of water intervening between the culture and the light, in order to obtain the maximum intensity of irradiation, and in no instance were the results altered. By referring to fig. 3, it is seen that these ineffective regions consist of wave lengths longer than 3000 Å.

A comparison of figs. 3 and 4 shows that spectrum regions containing wave lengths shorter than 3000 Å produce an inhibition progressively greater as the wave lengths become shorter. Probably the first inhibitory line is 2967 Å, from which point the irradiation becomes increasingly toxic. It should be noted, however, that possibly some of the mild inhibition under the chemical Pyrex filter may be due to the mercury arc line of 3024 Å, since it is excluded by the Blue Purple Ultra filter which gave no inhibition. In all instances, this inhibitory effect on the CO_2 elimination was detectable manometrically within five minutes.

It is interesting to note that the Red Purple Corex A, No. 986 filter passed radiations that are conspicuously more effective than did the Vita glass. This Corex glass differs qualitatively from Vita glass chiefly in its transmission of the strong line at 2537 Å. Irradiation of this wave length apparently is very toxic, since only about 5 per cent of its intensity calls forth an inhibition about 50 per cent greater than the combined effect of all longer wave lengths. It should be noted, however, that the Corex filter passes these radiations at a somewhat greater intensity than does Vita glass. The increase in area under the graph line (fig. 3), for wave lengths less than 3000 Å,

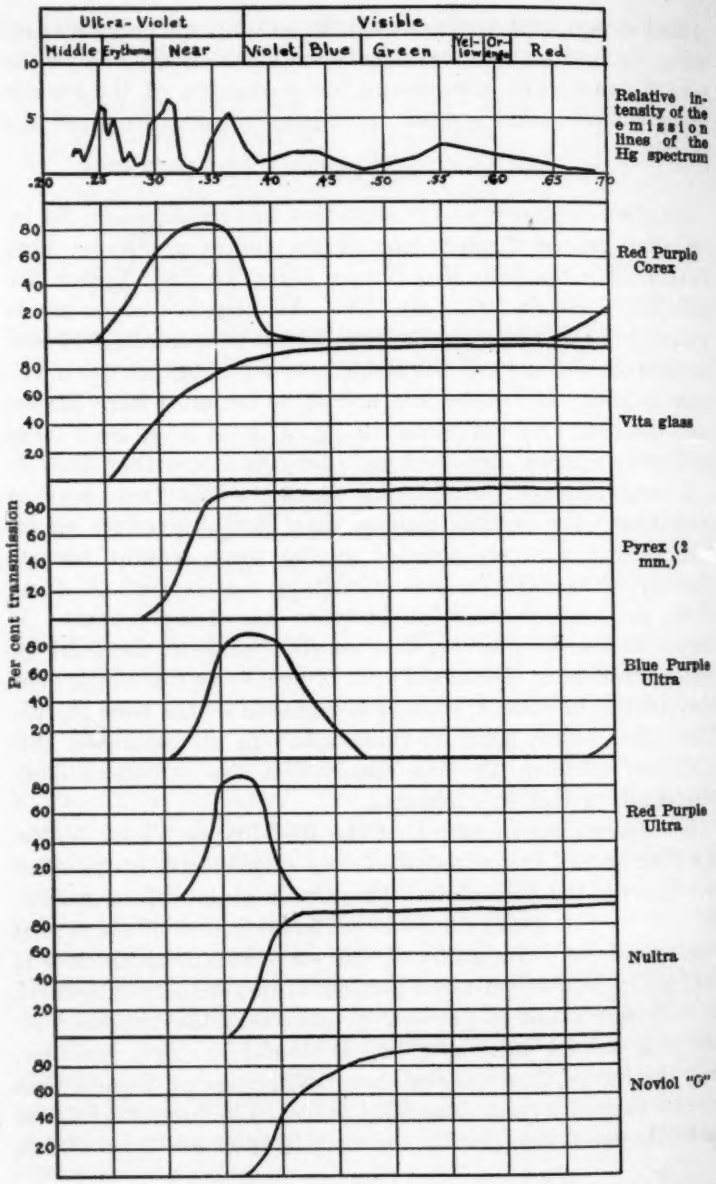

Fig. 3. Transmission of various filters.

for Vita glass as contrasted with Pyrex is about nine times, while the increase in inhibition is only about 60 per cent. This lack of proportionate relationship is probably due to the relatively low intensity of the radiations in this portion of the mercury arc. The corresponding comparison between Vita glass and the Corex filters is less than twice the area for the latter

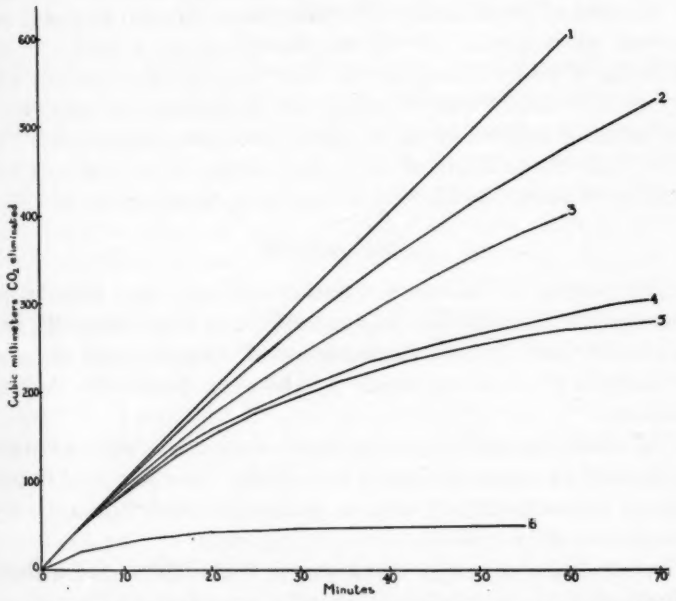

Fig. 4. Curve 1, Control; 2, irradiated through Pyrex and 4 cm. water; 3, irradiated through Vita glass and 4 cm. water; 4, irradiated through Red Purple Corex A and 1.5 cm. water; 5, irradiated through Red Purple Corex A and 4 cm. water; 6, irradiated by the open arc through 4 cm. water.

with an 80 per cent increase in inhibition. This close relationship is due no doubt in part to the relatively great intensity of the radiation of 2537 A. Thus it appears that this increased inhibition associated withe the Corex filter may be due to some extent to qualitative difference in the radiations transmitted and partly also to quantitative difference.

Curves 4 and 5 in fig. 4 are as close together as experimental error in the duplication of cultures would permit, and they in-

dicate that the difference in intensity produced by 4 and by 1.5 cm. of water was not sufficiently great to influence the results. Comparison of figs. 3 and 4 reveals that the inhibitory irradiation begins with the most effective erythemal region.

At the end of each experiment, the cells were treated with 1/2000 methylene-blue (final concentrations) to test for injured or dead cells. The unscreened arc produced complete staining at the end of 50 minutes of irradiation. No staining was observed when any of the filters described were used. If the staining of yeast by methylene-blue is accepted as evidence of injury this shows that the observed inhibition is a specific effect and not dependent upon a general injury to the cells. The inhibition due to death of the cells is much more profound and sudden as shown by the curve for the open arc in fig. 4.

CONCLUSIONS

The results of this investigation indicate that ultra-violet between 3000 and 2500 Å has an inhibiting effect upon the fermentative activity of suspensions of two-hour-old cultures of baker's yeast when made in the very favorable Pasteur medium.

This inhibition of fermentation is a specific effect and is not dependent on a general injury to the cell. The degree of inhibition of fermentation is both a qualitative and a quantitative function of ultra-violet.

Irradiations of longer wave length than 3000 Å are without effect.

A NOTE ON THE APPLICATION OF THE GLASS ELECTRODE TO THE DETERMINATION OF OXIDATION-REDUCTION POTENTIALS

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The electromotive force of the chain consisting of a glass electrode and a bare noble metal is influenced by the presence of oxidation-reduction systems in the solution. It has been frequently stated that such an electrode system may be used to determine oxidation-reduction potentials independently of simultaneous effects of pH, but this statement must be extensively qualified, as will be seen from the following discussion.

Ignoring the small asymmetry potential existing between the two glass surfaces, there are four sources of electrical activity in the chain. They are the half cell inside the glass electrode, the liquid-glass interface potential on the inner surface of the glass, the glass-liquid interface potential on the outer surface of the glass, and the oxidation-reduction potential imparted to the platinum. Any solution containing an oxidation-reduction system in which the glass electrode and the platinum electrode are immersed will affect the electromotive force of the chain in two ways. The oxidation-reduction system will impart a potential to the platinum, and the hydrogen ions present will modify the glass-liquid interface potential on the outer surface of the glass.

If the pH of the solution remains constant, then only changes in the oxidation-reduction potential will modify the E.M.F. of the chain. The true E_h of the oxidation-reduction system may be calculated by applying a calibration correction obtained by observing the E.M.F. of the chain when immersed in some known system at the same pH as that of the unknown

system. If the pH of the solution changes during the course of oxidation-reduction potential measurement, either of two conditions may exist. A consideration of the formula is necessary for their explanation:

$$E_h = E_o - \frac{RT}{nF} \ln \frac{(\text{reductant})}{(\text{oxidant})} - \frac{RT}{nF} \ln \frac{(\text{reductant})}{(\text{oxidant})} - \frac{RT}{nF} \ln \frac{K_a}{(H^+) + K_a}$$

The oxidation-reduction potential is seen to be a function of the ratio of total reductant to total oxidant, and also of pH.

First, if the system is such that the slope of the curve relating changes in pH to changes in oxidation-reduction potential is the same as that relating changes in pH to changes of the glass-liquid interface potential, then these two effects of pH cancel each other and the potentials of the system would correspond to those obtained at a constant pH. It is this that has given rise to the statement that the glass electrode records oxidation-reduction potential independently of pH changes. The falsity of this view comes from the fact that the observed reading must be corrected by an empirical factor obtained by observing the potential given by a known oxidation-reduction system at the same pH as that of the unknown. The magnitude of this calibration correction *changes* with pH, and hence its application to the observed reading will not give the E_h of the solution if the pH has changed from that of the calibrating system. In order to obtain the true E_h of a system of changing pH, determinations of pH must be made simultaneously with those of oxidation-reduction potential, from which new calibration corrections can be calculated.

On the other hand, if the system is such that the slope of the curve relating changes in pH to changes in oxidation-reduction potential is unlike that relating changes of pH on the glass-liquid interface potential, then the two effects of pH do not cancel each other. In order to convert observed potentials to E_h one would need to take simultaneous measurements of pH changes, and then apply as a correction factor only that part of the pH effect that is cancelled by the response of the glass electrode to the same pH changes. This would be a complicated

and tedious process—and only possible if the reaction of the redox system to changes in acidity were accurately known. Clark and Cohen[1] were the first to point out the various theoretically possible relationships between pH and oxidation-reduction potentials. They show how very complex is the effect of pH on many oxidation-reduction systems. For this reason, it is impossible to use this chain to determine the E_h of such complex biological systems as growing bacterial cultures, if the pH is changing simultaneously.

The use of the glass electrode in the determination of oxidation-reduction potentials is limited to buffered solutions, and its advantage lies solely in its high resistance and consequent lack of polarization. A high resistance (10 megohms) inserted in the usual calomel-platinum chain would be equally advantageous.

It may be pointed out that if the potential of the oxidation-reduction system changes with pH to the same degree as does the glass-liquid interface potential, then the observed E.M.F. of the oxidation-reduction system will be in terms of the pH *inside* the glass electrode. This apparently anomalous phenomenon has a simple explanation.

If the outside solution contains no oxidation-reduction system and is at the same pH as the fluid inside the glass electrode, then, ignoring the asymmetry potential, the potentials on the glass surfaces cancel each other, since they are functions of pH and are exerted in opposite directions. Again, for simplicity, ignoring certain possible ionic effects on the platinum, there remains in the chain but a single effective source of electromotive force, the half cell inside the glass electrode. Since a single source of electrical potential cannot be measured, there will be no E.M.F. detected in the system. Now, if quinhydrone be added to the outer liquid, a potential will be imparted to the platinum whose magnitude in comparison with the normal hydrogen electrode will be defined by the equation given above.

[1] Clark, W. M., and Cohen, B. Studies on oxidation-reduction. II. An analysis of the theoretical relations between reduction potentials and pH. U. S. Public Health Reports 38: 666–683. 1923.

The effective sources of electromotive force in the glass electrode-platinum chain consist of only the half cell inside the glass electrode and the oxidation-reduction potential imparted to the platinum, since the interface potentials on the glass surfaces cancel each other. The potential of the half cell inside of the glass electrode can be expressed in terms of the normal hydrogen electrode (E_h) by applying a suitable correction, and then the system reads the E_h of the oxidation-reduction system in terms, of course, of the pH, which under the conditions defined above is the same on both sides of the glass.

Now, if the pH of the solution in which the oxidation-reduction system is dispersed is changed by one pH unit, then the oxidation-reduction potential on the platinum changes by 59 millivolts (at 25° C.). However, this effect on the electromotive force of the oxidation-reduction system is the same quantitatively as that on the glass-liquid interface potential. These effects, therefore, cancel each other and the total E.M.F. of the chain will read as before. If the original pH equals 1.00 on both sides of the glass membrane, then it can be seen that no matter how the pH of the oxidation-reduction system outside be changed, the reading will still represent the E_h of the system at pH = 1.00. But if the pH outside the glass electrode remains at 1.00, and the pH inside is changed by 1 pH unit, the pH effect is no longer cancelled and the total E.M.F. of the chain will change 59 millivolts. The chain again does not indicate the E_h of the oxidation-reduction system, since that obviously has not changed. But the magnitude of this change, which is the pH effect on the inner surface of the glass, is the same as the change of oxidation-reduction potential if it were varied by this same pH unit.

It is now clear that the observed potential of all oxidation-reduction systems which vary 59 millivolts with a change of acidity of one pH unit will depend on the pH inside of the glass electrode, and curiously enough not that of the liquid in which the system is actually dispersed.

The validity of the above discussion depends on the assumption that the half cell inside the glass electrode is a metal in

equilibrium with its ions such as Ag: AgCl or Hg: HgCl. If this electrode is a noble metal in equilibrium with an oxidation-reduction system, e. g., platinum: quinhydrone, then the observed potential of the system will be the difference in millivolts between the oxidation-reduction potentials of the system inside and that outside the glass electrode, both dispersed in solutions of equal hydrogen ion concentration. Reconsideration of the above discussion will show that this will be true regardless of how the actual pH of the two solutions differ from each other, providing only that the pH of each is such that both systems vary 59 millivolts per pH unit change.

GENERAL INDEX TO VOLUME XXII

New scientific names of plants and the final members of new combinations are printed in **bold face** type; synonyms and page numbers having reference to figures and plates, in *italics;* and previously published names and all other matter, in ordinary type.

A

Adelosaccharomycetaceae, 349
Agalinis fasciculata, distribution of, in Missouri, *438*
Amblyanthera foliosa, 232, 238
Amelanchier canadensis in Schuyler Co., Mo., *756*
Anemone caroliniana, distribution of, in Missouri, *438*, of cylindrica, *437*
Angadenia Lindeniana, 232; Sagraei, inflorescence structure in, *17*, 18
Angadenia, 270; *Amazonica*, 279; *cognata*, 273; *coriacea*, 303; *Cururu*, 286; *elegans*, 303; *geminata*, 303; *grandifolia*, 286; *hypoglauca*, 301; *latifolia*, 279; *majuscula*, 301; *nitida*, 300; *Spruceí*, 279; *sylvestris*, 297
Animals: effect of ultra-violet, on basal metabolism of lower forms, 773, of human subjects, 781, of vertebrates, 775, on oxidizing enzymes of, 802
Anisolobus, 270, 271; *amazonicus*, 279, β. *latifolius*, 279; *anomalus*, 295; *cognatus*, 273; *Cururu*, 286, β. *grandifolius*, 286; *Fockei*, 286; *gracilipes*, 294; *hebecarpus*, 283, β. *glabratus*, 283, a. *tomentosus*, 283, a. *erectus*, 283, b. *scandens*, 283; *Hostmanni*, 286; *Kappleri*, 285; *lancifolius*, 306; *Perrottetii*, 288, β. *obtusus*, 288; *pulcherrimus*, 283; *puncticulosus*, 286; *Spruceí*, 279; *Zuccarinianus*, 283
Anthemis globosa, 102; *lutescens*, 112; *sinuata*, 104; *triloba*, 104; *trilobata*, 104
Apocynaceae: Observations on the inflorescence of, (with special reference to the American genera of Echitoideae), 1; schematic diagram of supposed relationship of certain types of inflorescence in, *39;* Studies in the, IV, 153
Apocynum, inflorescence structure in, 14; *Acouci*, 169; androsaemifolium, inflorescence structure in, 15, *15;* cannabinum, inflorescence structure in, 14, *15*, var. glaberrimum, 223; *paniculatum*, 169; *umbellatum*, 208
Apparatus: An improved, for the determination of carbon dioxide production in physiological plant studies, 361, *362*, 838; for raying yeast cultures, arrangement of, 854, *855;* A note on the application of the glass electrode to the determination of oxidation-reduction potentials, 861
Aptotheca, 153; *corylifolia*, 211
Arcangeliella **africana**, 365; **alveolata**, 365; **ambigua**, 365; **asterosperma**, 366, var. **depauperata**, 366, var. **hololeuca**, 366; **Beccari**, 366; **Behrii**, 366, var. **caudata**, 366; **brunneola**, 366; **Campbellae**, 366; *caudata*, 366; **cremea**, 367; **Curtisii**, 367; **ellipsoidea**, 367; **Gardneri**, 367; **glabrella**, 368; **krjukowensis**, 368, var. **michailowskjana**, 368; **laevis**, 368; **nana**, 368; **occidentalis**, 368; **pilosa**, 368; **scissilis**, 369; **socialis**, 369; **tasmanica**, 369; **violacea**, 369; **vulvaria**, 369.
Artemisia vulgaris var. gnaphalodes, distribution of, in Missouri, *438*
Arundinaria gigantea, distribution of, in Missouri, *437*
Asclepias perennis, distribution of, in Missouri, *439*
Aspergillus repens, 317, 326

B

Bacteria: effect of ultra-violet, on metabolism of, 790, on respirational enzymes of, 821
Balsamorhiza, 114; Bolanderi, 123; Careyana, 124; deltoidea, 125; *glabrescens*, 125; *helianthoides*, 127; hirsuta, 138, var. **lagocephala**, 139, var. **neglecta**, 139; **hispidula**, 137, *150;* Hookeri, 129, var. **lanata**, 130, var. **rosea**, 130; *Hookeri* var. *hirsuta*, 138; incana, 133, var. **tomentosa**, 134; in-

venusta, 122; **macrolepis**, 132; macrophylla, 134, var. **idahoensis**, 136; *macrophylla* var. *terebinthacea*, 135; **platylepis**, 131; *rosea*, 130; sagittata, 127; serrata, 136; terebinthacea, 140
Balsamorrhiza; 119; *Balsamorrhiza*, 129; *floccosa*, 133; *Hookeri* var. *incana*, 133; *tomentosa*, 134
Basal metabolism of animals, 773, of human subjects, 781, of lower forms, 773, of vertebrates, 775
Bean plants, effect of ultra-violet on respiration and respiratory enzymes of, 848
Berchemia scandens, distribution of, in Missouri, *437*
Biochemical studies with ultra-violet, 779
Bobartia, 88
Bostryx in Apocynaceae, 4, *39*
Botrytis, effect of ultra-violet on metabolism of, 792
Brauneria, 88; *angustifolia*, 91; *laevigata*, 91; *pallida*, 93; *paradoxa*, 94; *purpurea*, 89; *tennesseensis*, 92
Bumelia lanuginosa, distribution of, in Missouri, *440*
Buphthalmum sagittatum, 127

C

Calamagrostis canadensis var. Macouniana, distribution of, in Missouri, *438*
Cameraria Guyanensis, 252, 269; *lutea*, 252; *Tamaquarina*, 252
Carbohydrate reactions of Hæmispora coremiformis, 325, of Posadasia capsulata, 346
Carbon dioxide: elimination of, 853; an apparatus for the determination of, in physiological plant studies, 361, *362*, 853
Catalase, effect of ultra-violet on: of animals, 802, of bacteria, 821, of plants, 805, of bean plants, 849, of higher plants, 821, of tomato plants, 838
Catalogue, An annotated, of the flowering plants of Missouri, 375
Catalpa macrocarpa, 270
Chilophyllum, 100; *globosum*, 102
Cheilanthes alabamensis, in McDonald Co., Mo., *754*
Chrysanthemum americanum, 89
Chrysophania, 100; *fastigiata*, 112
Cincinnus in Apocynaceae, 4, *39*
Clathrogaster Beccari, 366; *vulvarius*, 369
Clematis Fremontii in Jefferson Co., Mo., *758*
Climate, irradiation as a factor of, 764, 784
Coccidioideaceae, 349
Coccidioides immitis, 348, 349
Codonechites, 270; *paniculata*, 305
Compositae, A critical study of certain epappose genera of the Helianthese-Verbesininae of the natural family, 51
Conidiophorales, 326
Coreopsis heterophylla, 81
Coutoubea, inflorescence structure in, 26
Cryptococcus, 335; farciminosus, 347, 349; muris, 349
Cunila origanoides, distribution of, in Missouri, *440*
Cycladenia Harrisonii, 297
Cylicadenia, 270; *Harrisii*, 297
Cytology of Geotrichum versiforme, 307

D

Dehydrogenase, effect of ultra-violet on: of animal tissues, 820, of plants, 821
Dichasium in Apocynaceae, 3, *39;* aggregate, 4, *39*
Dipladenia Harrisii, 297; *Harrisonii*, 297
Dodge, Carroll W., Sanford M. Zeller and. New species of Hydnangiaceae, 365
Dracunculus Virginianus latifolius, 89

E

Echinacea, 84; angustifolia, 91, var. tennesseensis, 92; *angustifolia*, 93; *Dicksonii*, 82; *dubia*, 81; *heterophylla*, 81; *intermedia*, 89; laevigata, 91; pallida, 93, *150;* paradoxa, 94; purpurea, 89; *sanguinea*, 91; *tennesseensis*, 93
Echites, inflorescence structure in, 11; crassipes, 13, *44;* turbinata, 11, *46;* turrigera, 13, *46;* tuxtlensis, 11, *46;* umbellata, 11, *44;* yucatensis, 11, *46*
Echites, 270; *amazonica*, 279; *arborea*, 249; *bicornis*, 279; *bracteata*, 202; *cognata*, 273; *coriacea*, 302; *corymbosa*, 218; *Cururu*, 285, var. α. *angustifolia*, 285, var. β. *grandifolia*, 285; *difformis*, 232, 234; *elegans*, 302; *ferruginea*, 232; *floribunda*, 214; *geminata*, 302; *gracilipes*, 294; *grandiflora*, 296; *hebecarpa*, 283; *Hoffmannseggiana*, 296; *hypoglauca*, 301; *lucida*, 300; *lutea*, 283; *macrantha*, 296; *macrocarpa*, 270; *Meyeriana*,

297; *nitida*, 300; *pilosa*, 159; *puberula*, 234; *pulcherrima*, 283; *puncticulosa*, 285; *salicifolia*, 234; *sessilis*, 269; *spicata*, 211; *stipellaris*, 295; *sylvestris*, 297; *thyrsoidea*, 200; *Vellosiana*, 202; *verrucosa*, 278; *Zuccariniana*, 283
Echitoideae, American genera of, 153; Observations on the inflorescence of Apocynaceae, with special reference to, 1
Elasmomyces **borneensis**, 370; **echinosporus**, 370; *russuloides*, 365
Electrode, glass, A note on the application of the, to the determination of oxidation-reduction potentials, 861
Endomycetaceae, 349
Enzymes: of animals, effect of ultra-violet on, 802; of higher plants, respiration and respiratory, Effects of ultra-violet on, 837, of beans, 848, of tomatoes, 837, 841
Erigeron pulchellus in Jefferson Co., Mo., *758*
Espeletia helianthoides, 127; *sagittata*, 127

F

Fagus grandiflora var. caroliniana, in low woods, Butler Co., Mo., *756*
Fat in cells of Geotrichum versiforme, 311
Ferdinanda, 100; *augusta*, 112, var. *megacephala*, 110; *lutescens*, 112; *montagnaefolia*, 108
Fermentation: effect of ultra-violet on, 790; of yeasts, Influence of various regions of the spectrum on the, 853
Fernaldia pandurata, inflorescence structure of, *48*
Flower, solitary, in Apocynaceae, 7, *39*
Forestiera acuminata, distribution of, in Missouri, *439*
Forsteronia, 153, inflorescence structure in 19; Acouci, 169; *acutifolia*, 161, *β pubescens*, 161; adenobasis, 207; affinis, 166; *Alexandri*, 214; amblybasis, 180, inflorescence structure in, *15*, 23; australis, 192; Benthamiana, 170; *bracteata*, 202; *brasiliensis*, 190, *β. bahiensis*, 190; brevifolia, 173; **chiriquensis**, 203; **cordata**, 186; *corylifolia*, 211; corymbosa, 218, inflorescence structure in, 19, *20*; **decipiens**, 181; *difformis*, 234; diospyrifolia, 175; Duckei, 177; elachista, 162, inflorescence structure in, *15*, 22; *emblioides*, 159; subg. **Euforsteronia**, 154; **fallax** Taub., 222; floribunda, 214, inflorescence structure in, 19; *floribunda*, 220; *foliosa*, 188; **galbina**, 198; Gardneri, 165; glabrescens, 194; gracilis, 158; **gracilioides**, 163; guyanensis, 172; *lagoënsis*, 220; laurifolia, 178; leptocarpa, 190, inflorescence structure in, *15*, *20*, 21; *leptocarpa* var. *glaberrima*, 190; Luschnathi, 161; *macrophylla*, 208; *meridionalis*, 159; *microphylla*, 202; *minutiflora*, 159; mollis, 187, var. **foliosa**, 188, var. typica, 188; montana, 183; *multinervia*, 200, *β glabriuscula*, 201, *γ microphylla*, 202, *δ ovalifolia*, 200; myriantha, 197; *obscura*, 201; obtusiloba, 193; *paludosa*, 179, inflorescence structure in, *15*, 22; *panniculata*, 223; *Pavonii*, 223; **peninsularis**, 215; pilosa, 159; portoricensis, 217, inflorescence structure in, 19; *protensa*, 161; subg. **Pteranthera**, 220; pubescens, 184; *pubescens β cordata*, 186; **Pycnothyrsus** K. Sch., 205; refracta, 200; *refracta* var. *contracta*, 221; Riedelii, 176; rufa, 167; *rufa* var. *subglabra*, 167; **Sandwithiana**, 209; *Schomburgkii*, 169, 208, *β umbellata*, 208; *Sellowii*, 183; simulans, 213, inflorescence structure in, *20*, 21; spicata, 211, inflorescence structure in, *15*, 23; **subcordata** K. Sch., 189; tarapotensis, 195; thyrsoidea, 199, inflorescence structure in, *20*, 21, var. **glabriuscula**, 201, var. typica, 200; **umbellata**, 208; Velloziana, 202, inflorescence structure in, *20*, 21; viridescens, 171; **Wilsonii**, 174
Fuller, H. J., F. Lyle Wynd, and Ernest S. Reynolds. Studies in ultra-violet and respiratory phenomena. II. The effects of ultra-violet on respiration and respiratory enzymes of higher plants, 837
Fungi: effect of ultra-violet on metabolism of, 790; yeast-like, 307, 317, 337

G

Geocarpon minimum, in Jasper Co., Mo., *750*
Geotrichum versiforme Moore, Cytology of, 307, *314*, *316*
Glass electrode, A note on the application of the, to the determination of oxidation-reduction potentials, 861

Glucose agar, growth of Geotrichum versiforme on, 308
Glutathione, 824
Glycogen in cells of Geotrichum versiforme, 311
Greenmaniella, 141; **resinosa**, 141, *152*
Growth in relation to external stimulus, 762
Gymnolomia scaposa, 83; *triloba*, 106
Gymnomyces Gardneri, 368; *pallidus*, 365

H

Haemadictyon grandiflorum, 297
Head infection caused by a new Hemispora, *H. coremiformis*, 317
Heliantheae-Verbesininae of the natural family Compositae, A critical study of certain epappose genera of the, 51
Helianthus invenustus, 122
Helichroa, 88
Heliopsis Balsamorrhiza, 129; *terebinthinacea*, 140
Hemispora, head infection caused by a new, 317
Hemispora, 326; **coremiformis**, 328, *332, 334;* pararugosa, 317, 326; rugosa, 317, 326; stellata, 317, 326
Hemisporosis, 317
Histoplasma capsulatum, 347, 349
Histoplasmaceae, 349
Histoplasmosis, 335
Hybridella, 100; *globosa*, 102
Hydnangiaceae, New species of, 365
Hydnangium **Archeri**, 371; *candidum*, 370; **citrinum**, 371; **Eisenii**, 371; **Gilkeyae**, 371; *glabrum*, 365; **Hessei**, 371; *hysterangioides*, 370; **laeve**, 372; **lanigerum**, 372; **luteum**, 372; **mistiforme**, 372; **monticola**, 372; **pusillum**, 372; *Ravenelii*, 367; **Soehneri**, 372; *tasmanicum*, 369; **Thaxteri**, 372; **tuberculatum**, 373
Hydrangea arborescens, distribution of, in Missouri, *440*
Hydrogen-ion concentration, relation of, to oxidation-reduction systems, 861
Hymenogaster *Behrii*, 366; *Campbellii*, 366; *luteus*, 373; *nanus*, 368; *violaceus*, 369; viscidus, 373
Hysterangium viscidum, 373

I

Infections, fungous, 307, 317, 337
Inflorescence of Apocynaceae: Observations on the, 1; structure of, *15, 17, 20, 24, 27, 30, 32*
Iostephane, 77; subg. **Chrysopetala**, 81; subg. **Euiostephane**, 80; heterophylla, 81, *150*, var. **acutiloba**, 83, var. **Dicksonii**, 82; trilobata, 83
Irradiation: as a climatic factor, 764, 784; ultra-violet from artificial sources, 781, reaction of animals to, 773, of human subjects, 781, of lower forms, 773, of plants, 759, 790, of bean plants, 848, of tomato plants, 760, 837, of yeast, 793, 853

J

Juniperus mexicana, on dolomite bluffs of White River, Barry Co., Mo., *746, 756;* virginiana, in Jefferson Co., Mo., *748*

K

Kallstroemia, A new, from Texas, 49
Kallstroemia **hirsuta**, 49, *50*

L

Laseguea leptocarpa, 190
Laubertia laxiflora, 305
Leitneria floridana, distribution of, in Missouri, *439*
Lepachys, 66; *columnaris*, 68, var. *pulcherrima*, 70, var. *Tagetes*, 72; *columnifera*, 68; *mexicana*, 76; *pedunculoris*, 73, var. *picta*, 74; *pinnata*, 75; *pinnatifida*, 75; *serratus*, 74; *Tagetes*, 72
Light, reaction of plants to, 760
Lipoidal substances in cells of Geotrichum versiforme, 311
Liquidambar Styraciflua, distribution of, in Missouri, *439*
Literature on ultra-violet and respiratory phenomena, Review of, 771
Lochnera rosea, inflorescence structure in, 31, *32*
Loess areas of Missouri, 429; plants of, 431; views in, *746, 752*
Lygodesmia juncea, distribution of, in Missouri, *437*

M

Macowanites *echinosporus*, 370; magnus, 369
Macrosiphonia, inflorescence structure in, 29, *30:* Brachysiphon, 30, *30;* hypoleuca, 29, *30;* longiflora, 31, *48;* Macrosiphon, 30, *30*

Malouetia, 238; *albiflora*, 253; amplexicaulis, 261; arborea, 249; *asiatica*, 269; cestroides, 248; cubana, 243; Duckei, 250; flavescens, 242; furfuracea, 254; *furfuracea β. grandifolia*, 255; glandulifera, 256; sect. **Graciles**, 263; gracilis, 263; **gracillima**, 265; guatemalensis, 267; *guianensis*, 269; *Guianensis*, 253; *jasminoides*, 269; Killipii, 262; *lactiflua*, 269; *lanceolata*, 249; lata, 245; *Martii*, 249; **Mexiae**, 246; nitida, 266; *obtusifolia*, 253; *obtusiloba*, 253; *odorata*, 252; *panamensis*, 267; **peruviana**, 259; *puberula*, 269; pubescens, 251; *retroflexa*, 243; *riparia*, 269; Schomburgki, 244, inflorescence structure in, *48*; *sessilis*, 269; Tamaquarina, 252, 269; *Tamaquarina β. brasiliensis*, 253, 256, *γ. Brasiliensis*, 249, *γ. lancifolia*, 253, *β. minor*, 252; sect. **Tamaquarinae**, 240; *Tarumensis*, 253; *tetrastachya*, 269; virescens, 264

Mandevilla: inflorescence structure in, 23, *24*, in Achrestogyne, 25, in bogotensis, 25, in callacatensis, *24*, 26; cuspidata, 306; foliosa, 232, 238; glandulosa, 306; scabra, inflorescence structure in, *24*, 26, in subpaniculata, 24, *24*, in xanthostoma, *24*, 25

Map of Missouri: divided into zones to show geographical range of species, *381*, showing the plant regions, *401*

Martellia mistiformis, 372

Media: growth of Geotrichum versiforme on various, 308; of Hemispora coremiformis, 322, of Posadasia capsulata, 340, of P. pyriformis, 343

Melanogaster ambiguus, 373, var. **eurysperma**, 373; *Eisenii*, 371

Metabolism, effect of ultra-violet on: of animals, 773, of human subjects, 781, of lower forms, 773, of vertebrates, 775; of plants, 790, of bacteria, 790, of Botrytis, 792, of yeast, 793

Milk, effect of ultra-violet on peroxidase activity of, 815

Missouri: An annotated catalogue of the flowering plants of, 375; annotated list of plants, 453; climate, 390; elevation and drainage, 387; flora, 398; geology and topography, 394; location and area, 385; map, showing geographical range of species, *381*; map showing plant regions, *401*; Ozark region, 406, views in, *746*, *756*, granitic-siliceous sub-region of, 411, sink-hole ponds, 409, White River sub-region and bald knobs of, 413, limestone and dolomite glades of, 422; prairie region, 427, glaciated sub-division of, 429, loess hills of, 431, *746*, *752*, unglaciated sub-division of, 431, views in, *746*, *752*, *756*; southeastern lowlands region, 400; tabular list of families, 683

Mitozus, 270; *gracilipes*, 294; *tenellus*, 300

Molopanthera paniculata, 223

Monilia, 307

Moore, Morris. Cytology of Geotrichum versiforme Moore, 307; Head infection caused by a new Hemispora: H. coremiformis, 317; A morphological and physiological study of two species of Posadasia, 335

Morphological and physiological study of two species of Posadasia, 335

Mycoderma, 307

N

Nectaromycetaceae, 349

Neogeotrichum pulmoneum, 348

Nyssa aquatica, distribution of, in Missouri, *439*

O

Obeliscaria, 67; *columnaris*, 68; *pinnata*, 75; *pulcherrima*, 70; *Tagetes*, 72

Octaviania africana, 365; *alveolata*, 365; *Archeri*, 371; *asterosperma*, 366, var. *depauperata*, 366, var. *hololeuca*, 366; *borneensis*, 370; *brunneola*, 366; *citrina*, 371; *compacta*, 370; *hysterangioides*, 370; *laevis*, 368, 372; *lanigera*, 372; *levis*, 368, 372; *lutea*, 372; *luteocarnea*, 370; *microsporium*, 366; *monticola*, 372; *occidentalis*, 368; *pusilla*, 372; *socialis*, 369; *tuberculata*, 373; *vacua*, 366

Octavianina alveolata, 365; *Hessei*, 371; *laevis*, 368, 372; *lanigera*, 372; *levis*, 372; *tuberculatum*, 373

Odontadenia, 270, inflorescence structure in, 16; affinis, 280; *amazonica*, 279; *angustifolia*, 300; subg. **Anisolobus**, 271; anomala, 295; sect. **Anomalae**, 295; *augusta*, 273; cognata, 273; *cordata*, 300; **cordigera**, 282; *coriacea*, 303; *Cururu*, 286; *cuspidata*, 306; *Dusenschoenii*, 300; subg. **Euodontadenia**, 296; *formosa*, 297; **funigera**, 274; geminata, 302; *glandulosa*, 306; glauca, 304; *goyazensis*, 294; **gracilipes**, 294; *gracilis*, 306; *grandiflora*, 297; *Harrisii*, 297;

Hoffmannseggiana, 296, inflorescence structure in, 16, *17*; sect. **Hoffmannseggianae**, 296; hypoglauca, 301; Killipii, 281; Kochii, 291; **lauretiana**, 289; laxiflora, 305, inflorescence structure in, *17*, 18; sect. **Laxiflorae**, 305; *lucida*, 300; lutea, 283; **neglecta**, 277; nitida, 300, α. *oblongifolia*, 300, β. *acuminata*, 300, γ. *angustifolia*, 300; sect. **Nitidae**, 299; Perrottetii, 288; *Poeppigii*, 303; polyneura, 304, inflorescence structure in, *17*, 18; puncticulosa, 285; Sandwithiana, 289; **Schippii**, 292; *speciosa*, 296; spoliata, 287; *Spruceі*, 279; stemmadeniaefolia, 299; **surinamensis**, 276; *sylvestris*, 297; verrucosa, 278; sect. **Verrucosae**, 271; *Zuccariniana*, 284, f. *angustifolia*, 284, f. *ovalifolia*, 284, var. *tomentosa*, 284

Oenothera missourensis in Barry Co., Mo., *758*

Oidium, 335; lactis, 307

Oospora, 307; d'Agatae, 327

Orthechites, 224; *Macnabii*, 231

Oxidation-reduction potentials, A note on the application of the glass electrode to the determination of, 861

Oxygenase, effect of ultra-violet on, 812, 839

Oxytropis plattensis, distribution of, in Missouri, *437*

Ozark region of Missouri, 406; characteristic plants of, 410; bald knobs, 415, plants of, 420; chert glades, 421, plants of, 422; granitic siliceous subregion, 411, plants of, 412; limestone and dolomite glades, 422, plants of, 422; views in, *746–758*; White River sub-region, 413

P

Palmer, Ernest J. and Julian A. Steyermark, An annotated catalogue of the flowering plants of Missouri, 375

Panicle in Apocynaceae, 6

Paracoccidioides, 349; brasiliensis, 348, 349

Parsonsia corymbosa, 218; *floribunda*, 214; *leptocarpa*, 190; *spicata*, 211

Pentstemon Cobaea var. purpureus, in Taney Co., Mo., *758*

Pericteria, 270; *stipellaris*, 295

Periploca umbellata, 218

Peroxidase, effect of ultra-violet on: of animal tissues, 815, of plants, 816, of beans, 848, of tomatoes, 839, 844, 847

Pinus echinata: along Little St. Francis River, Mo., *746*; in Shannon Co., Mo., *756*; distribution of, in Missouri, *440*

Populus tremuloides, distribution of, in Missouri, *438*

Posadasia, A morphological and physiological study of two species of, P. capsulata (Darling) Moore and P. pyriformis Moore, 335

Posadasia, 348; capsulata, 336, 340, 348, *352*, *354*, *356*; pyriformis, 336, 343, 348, *358*, *360*

Prairie region of Missouri, 427; glaciated sub-division of, 427, plants of, 429; loess areas, 429, *746*, *752*, plants of, 431; salt licks and saline springs, 431; unglaciated sub-division of, 431, plants of, 434; views in, *746*, *752*, *756*

Prestonia, inflorescence structure in, *27*, 28, in agglutinata, 28, *48*, in brachypoda, 28, in Meg'agros, *27*, 28, in mexicana, *27*, 28, in portobellensis, *27*, 28, in quinquangularis, 28

Protoglossum luteum, 373

Prunus virginiana, distribution of, in Missouri, *438*

Pteridium latiusculum, distribution of, in Missouri, *440*

Pteretis nodulosa, distribution of, in Missouri, *438*

Q

Quartz mercury arc, in irradiation experiments, 837, 843, 854, 857

Quercus falcata, distribution of, in Missouri, 437, of lyrata, 439, of Shumardii var. Schneckeii, 440

R

Raceme in Apocynaceae, 5, *39*

Ratibida, 66; columnaris, 68, *150*, var. *pulcherrima*, 70, f. **pulcherrima**, 70; *columnifera*, 68, var. *pulcherrima*, 71; **mexicana**, 76; peduncularis, 73, var. **picta**, 73; *picta*, 74; pinnata, 75; *sulcata*, 68; Tagetes, 71; *Tagetes* var. *cinerea*, 73

Reactions of plants to ultra-violet, 759

Respiration: of plants, apparatus for determining, 361; effects of ultra-violet on, of beans, 848, of fungi, 790, of green plants, 800, of tomatoes, 837, of yeast, 793, 853

Respiratory enzymes of higher plants, Effects of ultra-violet on respiration and, 800, 837

Respiratory phenomena, Studies in ultra-

violet and. I. Review of work published before June, 1935, 771; II. The effects of ultra-violet on respiration and respiratory enzymes of higher plants, 837; III. The influence of various regions of the spectrum on the anaerobic fermentation of yeast, 853
Review of ultra-violet work published before June, 1935, 771
Reynolds, E. S. The reactions of plants to ultra-violet, 759; and F. Lyle Wynd. Studies in ultra-violet and respiratory phenomena. I. Review of work published before June, 1935, 771; F. Lyle Wynd, Harry J. Fuller, and. *Ibid.* II. The effects of ultra-violet on respiration and respiratory enzymes of higher plants, 837; and F. Lyle Wynd. *Ibid.* III. The influence of various regions of the spectrum on the anaerobic fermentation of yeast, 853
Rhabdadenia Lindeniana, 232; *lucida*, 300; *polyneura*, 304
Rhinosporidium, 349; Seeberi, 349
Rhododendron nudiflorum var. roseum, in Madison Co., Mo., *758*
Robbia, 239; *cestroides*, 248; *gossipina*, 249; *macrocarpa*, 269
Rosa blanda, distribution of, in Missouri, *438*
Rudbeckia chrysantha, 83; *columnaris*, 68; *columnifera*, 68; *napifolia*, 81; *pallida*, 93; *pinnata*, 75; *purpurea*, 89; *serotina*, 89; *Tagetes*, 72

S

Sclerogaster **Broomeianus**, 370; **candidus**, 370; **hysterangioides**, 370; *lanatus*, 371; **luteocarneus**, 370; **pacificus**, 370; **siculus**, 371
Secondatia, 224; *arborea*, 249; densiflora, 225; *densiflora* α *genuina*, 225, var. *paraguariensis*, 225, β *parviflora*, 225; *difformis*, 232, 234; Duckei, 230; *ferruginea*, 232; floribunda, 229; *foliosa*, 229, β. *Gardneri*, 229, δ. *lanceolata*, 229, γ. *petiolaris*, 229; Macnabii, 231; peruviana, 227; Schlimiana, 228; *stans*, 232
Secotium (Elasmomyces) krjukowense, 368; *michailowskyanum*, 368
Sedum pulchellum, in Jasper Co., Mo., *758*
Sharp, Ward M. A critical study of certain epappose genera of the Heliantheae-Verbesininae of the natural family Compositae, 51
Silene Wherryi, distribution of, in Missouri, *440*
Smilax glauca var. leurophylla, distribution of, in Missouri, *437*
Southeastern Lowlands region of Missouri, 400
Spectrum: effect of various waves of, on plants, 759, 774, 838; The influence of various regions of the, on the anaerobic fermentation of yeast, 853
Splanchnomyces Behrii, 366
Sporendonema, 327; casei, 327; epizoum, 327
Stemmadenia grandiflora, 269; *guatemalensis*, 267
Steyermark, Julian A., Ernest J. Palmer and. An annotated catalogue of the flowering plants of Missouri, 375
Stimulation of plants under ultra-violet irradiation, 759
Studies in the Apocynaceae, IV. The American genera of Echitoideae, 153
Studies in ultra-violet and respiratory phenomena, I. Review of work published before June, 1935, 771; II. The effects of ultra-violet on respiration and respiratory enzymes of higher plants, 837; III. The influence of various regions of the spectrum on the anaerobic fermentation of yeast, 853
Sulphydryl compounds, effect of ultra-violet on, 824
Syringosma, 153

T

Tabernaemontana amygdalifolia, 269; *cestroides*, 248; *gracilis*, 263; *grandiflora*, 269; *laeta*, 249; *odorata*, 252; *populifolia*, 234; *riparia*, 269; tetrastachya, 269
Taxodium distichum: distribution of, in Missouri, *439;* in swampy woods, Mississippi Co., Mo., *756;* map showing northern limit of, *237*
Temnadenia, inflorescence structure in, 26, *27*, in stellaris, 26, *27*, in stenantha, 26, *27*, in violacea, 27, *27*
Temperature in relation to growth of plants, 762
Texas, a new Kallstroemia from, 49
Thalia dealbata, distribution of, in Missouri, *439*
Thenardia floribunda, inflorescence structure in, *48; corymbosa*, 208; *laurifolia*, 178; *umbellata*, 208
Thyrsanthus, 153; *Acouci*, 169; *adenobasis*, 207; *affinis*, 166; *Aubletianus*, 208; *Benthamiana*, 170; *bracteatus*,

202; *Brasiliensis*, 190; *corylifolia*, 211; *corymbiferus*, 208; *corymbosus*, 218; *crebriflorus*, 192; *difformis*, 234; *diospyrifolius*, 175; *embelioides*, 159; *Gardneri*, 165; *glabrescens*, 194; *gracilis*, 158; *Guyanensis*, 172; *laurifolius*, 178; *leptocarpus*, 190; *Luschnatii*, 161; *macrophyllus*, 208; *meridionalis*, 160; *multinervius*, 200; *myrianthus*, 220; *pilosus*, 159; *placidus*, 184; *populifolius*, 234; *pubescens*, 184; *rufus*, 167, *Schomburgkii*, 169; *spicatus*, 211; *Wilsonii*, 174
Thyrse in Apocynaceae, 6, *39*
Tomato plants, Effects of ultra-violet on respiration and respiratory enzymes of, 837; reaction of, to ultra-violet irradiation, 760
Torula epizoa, 327; sacchari, 327
Torulopsidaceae, 349
Trachelospermum, 232; asiaticum, 269; difforme, 232, 234, distribution of, *237*; jasminoides, 233; *stans*, 238
Trichosporum, 327
Tyrosinase, effect of ultra-violet on, 814

U

Ultra-violet: reaction to, of animals, 773, of bacteria, 790, of bean plants, 848, of Botrytis, 792, of plants, 759, of green plants, 800, of tomatoes, 760, of yeast, 793; and respiratory phenomena, Studies in, I. Review of work published before June, 1935, 771; II. The effects of ultra-violet on respiration and respiratory enzymes of higher plants, 837; III. The influence of various regions of the spectrum on the anaerobic fermentation of yeast, 853

V

Vaccinium vacillans var. crinitum, distribution of, in Missouri, *440*
Vacuoles in cells of Geotrichum versiforme, 311
Vinca minor, inflorescence structure in, 31, *32*
Vita glass in ultra-violet experiments, 842, 857
Volutin in cells of Geotrichum versiforme, 310

W

Williams, Louis. A new Kallstroemia from Texas, 49
Woodson, Robert E. Jr. Observations on the inflorescence of Apocynaceae (with special reference to the American genera of Echitoideae), 1; Studies in the Apocynaceae. IV. The American genera of Echitoideae, 153
Wynd, F. Lyle. An improved apparatus for the determination of carbon dioxide production in physiological plant studies, 361; A note on the application of the glass electrode to the determination of oxidation-reduction potentials, 861; and E. S. Reynolds. Studies in ultra-violet and respiratory phenomena. I. Review of work published before June, 1935, 771; Fuller, H. J., and Reynolds, E. S. *Ibid.* II. The effects of ultra-violet on respiration and respiratory enzymes of higher plants, 837; E. S. Reynolds and. *Ibid.* III. The influence of various regions of the spectrum on the anaerobic fermentation of yeast, 853

X

Xanthine dehydrogenase, effect of ultra-violet on, 822

Y

Yeast: anaerobic fermentation of, Influence of various regions of the spectrum on the, 853; effect of ultra-violet on fermentation of, 793; medium used, 856
Yeast-like fungi, 307, 317, 337
Yucca glauca, distribution of, in Missouri, *437*

Z

Zaluzania, 95; *angusta*, 112; anthemidifolia, 104; *asperrima*, 108, var. *montagnaefolia*, 108; augusta, 112; cinerascens, 109; *Coulteri*, 109; discoides, 107; subg. Ferdinanda, 101; globosa, 102, var. myriophylla, 103; *globosa*, 103; Grayana, 106; *Grayiana*, 106; subg. Hybridella, 101; megacephala, 110; mollissima, 110, *150*; montagnaefolia, 108; *myriophylla*, 103; nonensis, 113; Pringlei, 107; *quitensis*, 113; *resinosa*, 141; Robinsonii, 105; Sodiroi, 113; subcordata, 111; triloba, 104
Zeller, Sanford M., and Carroll W. Dodge. New species of Hydnaceae, 365
Zschokkea, 269